JOHN R. MURLIN HONOR VOLUME

VOLUME THIRTY-ONE

Through the special efforts of Dr. John R. Murlin, The Journal of Nutrition and a sponsoring organization, The American Institute of Nutrition, Inc., were established in 1928. In this venture Dr. Murlin had the assistance of ten leaders in the field of nutrition who served as members of an editorial board, namely, Eugene F. DuBois, Herbert M. Evans, Ernest B. Forbes, Graham Lusk, Elmer V. McCollum, Lafayette B. Mendel, Harold H. Mitchell, Mary S. Rose, Henry C. Sherman and Harry Steenbock. Arrangements were made for Charles C. Thomas of Springfield, Illinois, to serve as the publisher. The new Journal grew steadily in its acceptance among scientific workers and in its circulation. With respect to the latter, however, the growth was not sufficient to meet the deficits faced by the publisher. Dr. Murlin finally arranged for The Wistar Institute of Anatomy and Biology in Philadelphia to undertake its publication. This move on the one hand placed the resources of The Wistar Institute and its printing facilities behind the Journal, and on the other hand gave to The Wistar Institute and its long list of distinguished scientific periodicals representation in the field of nutrition. The original sponsoring organization, The American Institute of Nutrition, Inc., was also changed to a scientific society whose members are active investigators in the field of nutrition. This organization was eventually admitted to membership in the Federation of American Societies of Experimental Biology.

In 1939, when he had reached the age set for emeritus membership in the society, Dr. Murlin resigned his editorship. He continued at his post as Professor in the University

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of Rochester until 1944 when he reached the retirement age of 70 years. Because the war had taken away so many of the younger members of the staff, Dr. Murlin was asked by his University to continue his services for a period which finally ended in June, 1945.

It is fitting that this Journal honor Dr. Murlin for the part he played in establishing it and in guiding it through its early years. This recognition takes the form of designating volume thirty-one, which begins with this issue, as the *John R. Murlin Honor Volume*. An article of appreciation written by one of his younger colleagues accompanies the photograph which serves as a frontispiece for this volume.

G. R. C.

JOHN RAYMOND MURLIN

INVESTIGATOR, TEACHER, COLLEAGUE

An Appreciation

It is well known that the experiences of childhood have much to do in shaping the character of a man. There are reasons for believing this to have been the case with the lad who was destined to become known as John R. Murlin, distinguished American investigator and teacher of physiology and nutrition.

In his early days in Ohio, young Murlin was occupied as assistant to his father, who operated a village store as well as a nearby farm. They were busy days filled with the marvelous variety of tasks and interesting adventures known only to the boy who grows up in the country. The environment of his youth accounts, in no small way, for the fact that he has always "kept his feet on the ground." After completing a normal school course he became a country schoolmaster for a time in order to earn enough money to pursue his formal education further. He was awarded the B. S. degree in 1897 and the M. A. 2 years later by Ohio Wesleyan University where his major interests were biology and chemistry. Professor Conklin, the eminent zoologist from the University of Pennsylvania, on a visit to Ohio Wesleyan, met the enthusiastic young biologist who was then an instructor in charge of two courses in physiology. The result of this meeting was the offer of a fellowship in Conklin's department to the young man who was so eager to extend his scientific horizons and in 1901 Pennsylvania conferred on him the Ph. D. degree. His thesis was on digestion and absorption in the land isopods, indicating a manifest predilection for the functional rather than the structural aspects of his subject.

As a college student John Murlin met and fell in love with Josephine Seaman and they were married in 1899 while he was still a graduate student in Philadelphia. "Now it can be told" that she was taking a course in physiology at Ohio Wesleyan which, on account of the sudden illness of the regular instructor, her future husband was asked to teach, and that she withdrew immediately when she realized the embarrassment which her presence elicited in the substitute instructor. This discerning solicitude for the man of her choice has continued and grown for nearly half a century.

John Murlin was appointed Professor of Biology and Instructor in Chemistry at Ursinus College, a position which he held until 1903 when the opportunity of a lifetime presented itself in the form of an instructorship in the laboratory of the great Graham Lusk.

The new instructor came to Lusk's laboratory with definite ideas concerning his own research program — he wished to study the metabolism of proteins. Lusk, trained in the best German tradition, must have been somewhat taken aback by the confidence of the relatively inexperienced newcomer who insisted on independent investigation, but the Professor was persuaded and suggested a study of the nutritive value of gelatin which at that time was being much discussed as a substitute for meat. While engaged on this project, Murlin found time to translate, from the German, Tigerstedt's "Textbook of Human Physiology." Following the work on gelatin came a beautifully planned and executed investigation on the nitrogen and energy metabolism of pregnancy in dogs at Cornell, and in humans at the Nutrition Laboratory in Boston with Thorne Carpenter. Later more work appeared on human pregnancy and the metabolism of infants in collaboration with H. C. Bailey and with B. R. Hoobler. This work brought invitations to write the chapter on metabolism in Abt's "Pediatrics," and to deliver the Harvey Society lecture in 1917.

Ever since von Mering and Minkowski demonstrated pancreatic diabetes in 1889 many investigators had been in-

trigued by its similarity to diabetes mellitus in man and the possibility of discovering a therapy adequate for its control. Murlin, too, was attracted to this problem and proposed his scheme of attack to Professor Lusk, whose comment was, "Oh, but Minkowski tried that and failed." The work was undertaken, nevertheless, and in 1913 Murlin and Kramer published strong evidence that their extracts of pancreas were active. The diabetic dog, in many instances, responded to extract injection with increased R. Q. and diminished glycosuria and indeed in one experiment the urine remained free of reducing substances for 4 hours. In control experiments it appeared that the weak alkali, used to neutralize some of the extracts prior to administration, on injection gave results similar to those obtained with the extract itself. Much time was spent seeking an explanation of this unlooked for complication and the work was interrupted by our entry into World War I.

John Murlin was by strong conviction and family tradition impelled to take an active part in the war and, on leave of absence from Cornell, volunteered for officer training in the summer of 1917. His observations on the Army ration at Plattsburg training camp, with his practical suggestions for its improvement, soon brought him to the Surgeon General's Office in Washington as a major in the Sanitary Corps and an assignment to organize the Nutrition Division. This was accomplished with characteristic vigor and dispatch and for the first time the Army learned the actual food consumption of trainees under various conditions as well as the extent of food wastage. The data collected by Major Murlin, and some three score officers whom he brought into his organization, pointed the way to improvement of the ration on a nutritional basis rather than the traditional method of merely "filling the cavity" when a soldier got hungry. The overall result of this pioneer work was to awaken in the Quartermaster Department a broadened interest in its responsibility for feeding the soldier and in the Medical Department a new conception of its responsibility for his nutritional status. The

success of the Nutrition Division in the Army, where rights and responsibilities are rigidly prescribed by regulation and any threat to usurpation of prerogative is quickly eliminated, testifies to the versatility and adroitness of its director. He was promoted to a Lieutenant Colonelcy and finally separated from the service in the Spring of 1919.

Lewis P. Ross, former president of the board of trustees of the University of Rochester, bequeathed the greater portion of his estate to endow, " . . . a Department of Vital Economics which shall conduct instruction and experimentation in physiology, hygiene and nutrition of the human body to the end that human life may be prolonged with increased health and happiness." In 1916 John Murlin was invited to organize and direct the newly endowed department but declined because the university authorities were unsympathetic toward the newfangled idea of research. A year later, while he was still an officer candidate at Plattsburg, the offer was renewed and accepted with a clear understanding that the " . . . experimentation . . . " provided for in the terms of the bequest should receive its full share of attention. The University granted him leave of absence for the duration of the war, and he went off to the Surgeon General's Office in Washington. Despite his heavy responsibilities in the Army, the new director of the laboratory was able, by occasional hurried trips between Washington and Rochester, to plan and supervise an investigation on the antiscorbutic potency of dehydrated fruits and vegetables.

On completion of his Army service, he returned to Rochester to take active charge as Director of the Department of Vital Economics and Professor of Physiology. The staff was increased and graduate students began to arrive. Naturally, work was resumed on some of the prewar projects, including pancreatic diabetes, the metabolism of children and protein metabolism. The anti-diabetic substance was destined to be unequivocally demonstrated in another laboratory and Professor Murlin, concealing any personal regret he may have felt at having been unsuccessful in doing

the crucial experiments, joined scientific workers everywhere in acclaiming the magnificent achievement of Banting and Best. Their announcement was a stimulus to greater activity in Rochester and many papers appeared on the preparation, physiological effects and chemical properties of insulin.

In the quarter century since Professor Murlin took active charge, the Department of Vital Economics has accomplished a prodigious amount of work. Limitations of space do not permit even a listing of the papers that have been published, 220 odd, much less comments on them and the names of all the people who worked in the laboratory. The work in endocrinology broadened to include hormones of the thyroid, pituitary, adrenals, gonads and digestive tract. In a study of gluconeogenesis from fat a new calorimeter was devised which gave better agreement between direct and indirect heat measurements in man than any other apparatus hitherto described. The interest in proteins was intensified and extended and many papers were published on biological values as well as the protein sparing effect of various carbohydrates. The culmination of a long and active interest in protein metabolism came, appropriately, in the year preceding Professor Murlin's retirement when he was able with human subjects to characterize the biological values of certain proteins in terms of their constituent essential amino acids. A great deal of the work was done using human subjects and is a significant contribution not only in the placing of proteins in nutritional perspective but in the technique of nutritional investigations in man.

The immediate problems of the laboratory were not the only concern of its director. From 1919 to 1922 he was chairman of the Committee on Food and Nutrition of the National Research Council and again served as a member from 1941 through the greater part of World War II. In 1932 he was a member of the White House Conference on Child Health and Development and a delegate to the international conference on nutrition in Berlin sponsored by the Health Division of the League of Nations. He early saw the need for a journal

devoted especially to the publication of fundamental investigations in nutrition, and was the prime mover in the establishment of The Journal of Nutrition, which he edited through its first seventeen volumes (1928–1939); he had a most prominent part in organizing the Institute of Nutrition and is the only one of its members that has served two consecutive terms as president. The American Philosophical Society elected him to membership in 1932.

As a teacher of graduate students, Professor Murlin was firm in his insistence on a thorough grounding in the history and principles of his subject. He took great pains to present in seminars and lectures the development of the science of nutrition. When students reported on the classical works of Lavoisier, Liebig, Voit, Rubner and Lusk the Professor always listened eagerly to learn whether the neophyte had grasped the significant points and he was always ready to steer the discussion in order to correct misinterpretations and supply omissions. This he did easily and entertainingly because he was conversant not only with the details of the paper under discussion but often illuminating personal characteristics or incidents concerning its author. The historical aspect of science in general was pursued further at monthly Sunday evening meetings in the Murlin home. Here the whole department, wives included, enjoyed a buffet supper and social hour before the evening's reading was begun by some member of the department. These delightful intellectual excursions took one into the realms of chemistry, physics, medicine and even mathematics and astronomy. Mrs. Murlin, besides providing for the immediate nutritional needs of the "family", always took an active part in the reading and discussion. The memories of these gatherings in the gracious and cultured home of the Murlins are cherished by all who were privileged to participate.

In the laboratory there was great freedom of thought and action, for all who deserved it. Professor Murlin was vehemently opposed to pouring all students into the same mold. From the beginning of his own career with Lusk, he insisted

on independent investigation for himself and, after he became director of the laboratory at Rochester, he consistently encouraged students to embark on independent investigations as soon as they were ready for such ventures. As a logical consequence of this policy he refrained from attaching his name to every paper that came out of the laboratory just because he happened to be the director. He was exceptionally generous in this regard and shared the work as well as the credit in any research the results of which were published under joint authorship. Each new candidate began his post-doctorate career as the sole author of at least one paper. This represents a definite break in the traditional method of graduate instruction largely inherited from the European, and especially the German universities where the professor was often an autocrat, allegedly omniscient, who dominated a student's thinking and dictated his every move. It is a paradox still, in some American universities, that preaching the democratic ideal of freedom far outruns its practice.

The early recognition and encouragement of talent in investigation was considered fundamental. Students were urged to present results of their work before local and national meetings, and in the post-doctorate period, as soon as subsequent work confirmed the Professor's estimate of a man's capacity for productive scholarship, he was strongly supported in his application for membership in the appropriate professional society. Without being uncritical, Professor Murlin held no brief for exclusiveness in the fraternity of science.

Great devotion to the ideas and ideals of science is not always easy. One of his colleagues has said that among Professor Murlin's many achievements one of the most noteworthy was the initiation and courageous maintenance of a research program in an environment that at best was indifferent and often was downright hostile. How different the situation is a quarter of a century later! With the opening of the School of Medicine in 1925 and the advent of its staff of young and eager investigators it became much easier and even

fashionable to indulge in research. At present it is exceptional for any staff member not to be engaged in research and Professor Murlin stands in the van of the pioneers who brought about this complete change of attitude.

His exuberant initiative and vigorous activity were scarcely contained in over 4 decades of scientific endeavor; they overflowed into his recreation. The grounds at the Murlin home with their expanse of lawn, lovely trees and beautiful gardens give eloquent testimony to the inspiration and expert care of a master gardener. Students and colleagues frequently were invited to spend an afternoon at tennis, baseball, horseshoes or just relaxing in the shade waiting for supper al fresco. The Murlins are very fond of travel and have managed to see most of the United States and large portions of Europe, Canada and Mexico. When color film became available, Professor Murlin brought home most fascinating motion pictures of far places and peoples which, with Mrs. Murlin's lovely water colors, illustrated many an interesting travelogue of a Sunday evening. The Professor liked to motor to scientific meetings and the car was always filled with colleagues or students from the laboratory. His keen delight in exploring new places and his extensive knowledge of the flora, fauna and history of the regions visited made these trips rare occasions of high adventure.

On June 30, 1945, at age 71, John Murlin retired as Lewis P. Ross Professor of Physiology and Director of the Department of Vital Economics. He had generously agreed to defer his retirement a year on account of the depletion of the staff occasioned by the war. In his final year he probably worked harder than at any other time in his career at Rochester. He was an indefatigable worker up to the last day of his active duty and will doubtless continue in the same manner in whatever he chooses to do in the future. His constant vigorous attack on current problems was always a challenge to students and colleagues. Few achieved his pace but all were better for the attempt.

E. S. N.

THE DETERMINATION OF METABOLIC FECAL NITROGEN AND PROTEIN DIGESTIBILITY

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TWO FIGURES

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During the course of an investigation of the nutritive quality of proteins for growth (Bosshardt et al., '45) it was necessary to determine the "true digestibilities" of the proteins studied. This involved the estimation of the fecal nitrogen of non-dietary origin (metabolic fecal nitrogen) during the period of active growth.

At present, two general methods are available for the determination of the metabolic fecal nitrogen. The determination of the fecal nitrogen excretion of animals fed a nitrogen-free diet can be determined directly and has been used by some workers (Mitchell and Carman, '24) as a metabolic fecal nitrogen value, The inability of animals to grow and, with the smaller species such as the rat and the mouse, to maintain a normal food consumption on a nitrogen-free ration makes this method of limited use. Mitchell and Carman ('24) have indicated that whole egg protein is completely digestible. It has since become a practice of some investigators to incorporate a small amount of whole egg protein in the diet, usually 4 to 5%. The total fecal nitrogen excreted, assuming complete digestion of the whole egg protein, then would represent the metabolic fecal nitrogen. Neither of these techniques is applicable to the measurement of "true digestibility" during a prolonged period of growth.

Titus ('27) determined the "true digestibity" of alfalfa protein in steers by means of a different technique. The protein content of the ration was varied and the total food intake was maintained constant. The total nitrogen intake was plotted as a function of the total fecal nitrogen excretion. The resulting straight line was extropolated to the point of zero nitrogen intake, the Y intercept then being used as the metabolic fecal nitrogen excretion. The suggestion was made that in steers, the fecal nitrogen excretion on a nitrogen-free diet could not safely be taken as a measure of metabolic fecal nitrogen when the animals were ingesting protein.

Schneider ('35) has shown that the metabolic fecal nitrogen fraction consists of two components: a constant fraction that is proportional to the body surface area, and a variable fraction that is proportional to the quantity of dry food consumed. The variation due to differences in the quantity of food consumed is corrected for by expressing metabolic fecal nitrogen as a function of food consumption, i.e., mg. of metabolic fecal nitrogen per 100 gm. of food or dry matter consumed.

The object of this study was to investigate an extropolation procedure for estimating metabolic fecal nitrogen, using mice as test animals.

EXPERIMENTAL METHOD

Male albino mice (Sharp and Dohme, Swiss-Webster strain) were used in these studies. In the one study involving restricted feeding mature mice weighing from 20 to 30 gm. were used. In the experiments involving growth, mice weighing 7 to 9 gm. at 15 to 16 days of age were selected and subjected to a preliminary adjustment period of 2 days (Bosshardt et al., '45). In all experiments, the mice were housed in individual wire-bottom cages and supplied water ad libitum. The feces for each group were pooled for the entire test period in 10% sulfuric acid. Nitrogen determinations were made by a modified Kjeldahl procedure in which the total sample was di-

gested partially by refluxing with 20% sulfuric acid after which aliquot portions were used for the final digestion and distillation.

The protein sources studied were: acetone extracted, heat coagulated whole egg, casein,[1] and wheat gluten. They were incorporated at the different levels into a basal ration that consisted of 25% hydrogenated cottonseed oil (Primex), 2% corn oil,[2] 20% dextrose,[3] 4% salt mixture (Hubbell, Mendel, and Wakeman, '37), 2% cellu flour, 1% Wilson's 1:20 liver concentrate powder and sufficient white dextrin to make 100%. All diets were supplemented to contain per 100 gm.: 4 mg. of alpha-tocopherol, 900 U.S.P. units of vitamin A, 180 U.S.P. units of vitamin D, 1 mg. of 2-methyl-l, 4-naphthoquinone diacetate, 0.8 mg. of thiamine hydrochloride, 1.6 mg. of riboflavin, 0.8 mg. of pyridoxine hydrochloride, 4.0 mg. of niacin, 4.4 mg. of calcium pantothenate, 4.0 mg. of para-aminobenzoic acid, 21.6 mg. of inositol, and 200 mg. of choline chloride. In the study with restricted food intake, the liver concentrate powder was omitted.

Digestibility with restricted food intake

The data in this experiment were obtained in a study undertaken to determine the effect of protein feeding on body protein loss when mice were restricted to approximately 30% of their normal caloric intake. The diets contained extracted whole egg at eight levels ranging from 0% to 40% (table 1). Each diet was fed to a group of eight mice in an amount of 1.0 gm. per mouse per day for 14 days.

The relationship between nitrogen intake per 100 gm. of food consumed and fecal nitrogen per 100 gm. of food consumed is shown in figure 1. As plotted, the data, with the exception of that obtained with the protein-free diet

[1] Borden's "Labco" casein.
[2] Mazola.
[3] Carelose.

approximate a straight line. The straight line of best fit was calculated from the equation, $Y = a + b$ where

$$b = \frac{[\Sigma (xy)]\, n - \Sigma x \cdot \Sigma y}{[\Sigma (x^2)]\, n - [\Sigma (x)]^2} \quad \text{and} \quad a = \frac{\Sigma y - b\Sigma x}{n} \quad \text{or} \quad a = \bar{y} - b\bar{x}$$

The standard deviation of *a* was calculated according to the equation

$$\sigma a = \sqrt{\frac{\Sigma x^2 (\Sigma y^2 - a \cdot \Sigma y - b \cdot \Sigma xy)}{n\,[n - 2 (\Sigma x^2 - n\bar{x}^2)]}}$$

The value obtained with the protein-free diet was omitted from the calculation because of its marked deviation from the straight line, a finding that tends to confirm the observation of Titus ('27) that the fecal metabolic nitrogen excretion on a protein-free diet may not be the same as when protein is being consumed.

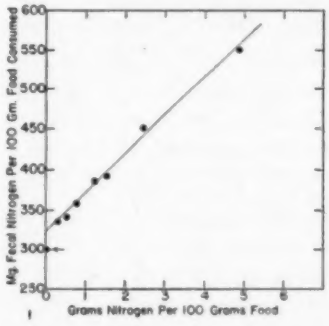
The extropolation of this line to the point of zero nitrogen intake gave a value of 322.9 mg. of metabolic fecal nitrogen per 100 gm. of food consumed. The value obtained with the protein-free diet was 300 mg. per 100 gm. of food consumed. The standard deviation of the extropolated value was $\pm$ 3.7. This is an indication that with mice the metabolic fecal nitrogen is affected by the feeding of protein and erroneous results may be obtained by using a value yielded when protein-free diets are fed. The calculated "true digestibility" of the whole egg protein, using the correction of 322.9 mg. of fecal nitrogen per 100 gm. of food consumed for this experiment, was 95.4 $\pm$ 0.2%.[4]

Digestibility with ad libitum food intake

The relationships between nitrogen intake and fecal nitrogen per 100 gm. of food consumed for mice ingesting the three proteins ad libitum are shown in figure 2. The metabolic fecal nitrogen values obtained by extropolation were: whole egg, 221 mg. per 100 gm. of food consumed, and for wheat gluten, 217 mg. per 100 gm. of food consumed. The data for the

[4] Standard error $= \sqrt{\frac{\Sigma d^2}{n\,(n-1)}}$

casein-fed animals included one anomalous value — that obtained with the lowest level of nitrogen intake. The fecal nitrogen per 100 gm. of food consumed actually found by analysis was 189 mg., whereas the value calculated from the straight line was 244 mg. The standard deviation of the Y intercept was found to be ± 7.1 which is an indication that the observed difference was highly significant. This value therefore was omitted in the calculations of the Y intercept and average digestibility.



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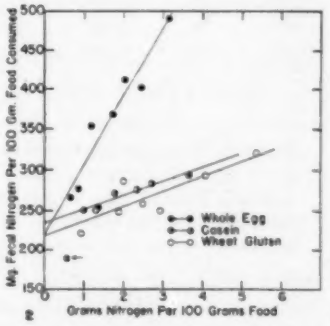

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Fig. 1 The relationship between nitrogen intake and fecal nitrogen excretion with varying levels of whole egg protein in the diet. The food intake was restricted to approximately 30% of the normal caloric requirement.

Fig. 2 The relationship between nitrogen intake and fecal nitrogen under conditions of ad libitum feeding with varying protein levels in the diet.

The casein-fed group showing the anomalous result did not grow throughout the feeding period. If, as these data suggest, the metabolic fecal nitrogen when the animal is just in nitrogen equilibrium is not the same as when the animal is in positive nitrogen balance, as is the case during growth, values obtained under one set of conditions are not necessarily adaptable to an entirely different set of conditions.

The "true digestibilities" at the different levels of intake of the proteins studied are shown in table 1. The two sets of data obtained with whole egg feeding confirm the results of Barnes et al. ('45), who reported an increase in digesti-

TABLE 1

Protein digestibilities with varying levels of intake

WHOLE EGG-RESTRICTED [1]		WHOLE EGG AD LIBITUM [2]		CASEIN AD LIBITUM [3]		WHEAT GLUTEN AD LIBITUM [4]	
N intake per 100 gm. diet [5]	"True digestibility"	N intake per 100 gm. diet	"True digestibility"	N intake per 100 gm. diet	"True digestibility"	N intake per 100 gm. diet	"True digestibility"
gm.	%	*gm.*	%	*gm.*	%	*gm.*	%
0.287	95.5	0.660	92.9	1.000	98.4	0.920	99.8
0.532	96.4	0.870	93.4	1.360	98.7	1.300	97.6
0.770	95.5	1.190	88.7	1.780	98.0	1.870	98.9
1.220	94.9	1.730	91.4	2.350	98.3	2.000	96.6
1.540	95.5	2.020	90.4	2.710	98.2	2.480	98.4
2.480	94.9	2.440	92.5	3.630	98.4	2.930	98.9
4.890	95.4	3.140	91.3	..	..	4.080	98.2
..	..	..	..	..	..	5.370	98.1
	95.4 ± 0.2 [6]		91.5 ± 0.6		98.3 ± 0.1		98.3 ± 0.3

[1] Metabolic fecal nitrogen = 323 mg./100 gm. food consumed.
[2] Metabolic fecal nitrogen = 221 mg./100 gm. food consumed.
[3] Metabolic fecal nitrogen = 235 mg./100 gm. food consumed.
[4] Metabolic fecal nitrogen = 217 mg./100 gm. food consumed.
[5] Equivalent to per cent nitrogen in the diet.
[6] Standard error = $\sqrt{\frac{\Sigma d^2}{n\,(n-1)}}$

bility when the protein intake was restricted by paired feeding as compared with ad libitum feeding.

DISCUSSION

In studies of protein nutrition an estimation of the "true digestibility" often is desired. It is recognized that nitrogen in the feces originates from two sources: unabsorbed food nitrogen and nitrogen resulting from body metabolism and excreted by way of the feces. The fecal nitrogen excretion on a protein-free diet frequently has been used as a measure of the metabolic fecal nitrogen. This is based on the assumption that the metabolic fecal nitrogen is dependent upon body surface area and the amount of dry food consumed and is not influenced by the presence of protein in the food. The extrapolation procedure is an attempt to determine the metabolic nitrogen of the feces under conditions of protein feeding.

The data in this report indicate, as do the results of Titus ('27), that the fecal nitrogen excretion on a protein-free diet is not a safe measure of metabolic fecal nitrogen when protein is included in the diet.

When the nitrogen intake per unit of food consumed at several different levels of intake is plotted as a function of the fecal nitrogen per unit of food consumed, the plot of the data fits very well a straight line. If a protein is completely digested and absorbed this line should be parallel to the X axis. As the slope of this line increases with different proteins, the "true digestibility" decreases.

The data indicate that whole egg protein is not completely digestible, a finding that is contrary to the results of Mitchell and Carman ('24). This may be due to a species difference between the mouse and the rat; however, the results of Barnes, Maack, Knights and Burr ('45), which were obtained with rats, indicate that wheat gluten is digested more completely than is whole egg protein.

Another interesting point is the increase in "true digestibility" of egg protein when the food intake is restricted. This appears to be similar to other cases in which the degree and rate of absorption is influenced by the state of deficiency or it may be due in part to the peculiar absorption pattern that is found when animals consume their daily food allotment in a comparatively short time.

It follows from the work of Schneider that the metabolic fecal nitrogen when expressed as a function of food consumed should increase when food consumption is restricted. This has been explained as evidence of a second factor that involves intestinal secretion and is related to body size. The observation that a straight line relationship exists between the nitrogen content of the ingested food and the fecal nitrogen per unit weight of food consumed indicates that there is no serious deviation in the relative contribution of these two factors toward the total metabolic fecal nitrogen under conditions of protein feeding. However, under conditions of severe protein restriction or the ingestion of a protein-free diet it is possi-

ble that the intestinal secretion of nitrogen is markedly decreased. This would result in an apparent lowering of the metabolic fecal nitrogen.

SUMMARY

"True digestibilities" of the proteins of whole egg, wheat gluten, and casein and the fecal nitrogen of metabolic origin have been determined under conditions of protein feeding with growing mice. The metabolic fecal nitrogen was determined by plotting the nitrogen intake per unit of food consumed at different dietary protein levels as a function of the fecal nitrogen excretion per unit of food consumed. The calculated straight line was extropolated to the point of zero nitrogen intake. The Y intercept was then used as the value for the metabolic fecal nitrogen per unit of food consumed.

Under conditions of ad libitum feeding the average "true digestibilities" determined by this method were: casein 98.3 $\pm$ 0.1%, wheat gluten 98.3 $\pm$ 0.3%, and extracted whole egg 91.5 $\pm$ 0.6%. When the food intake of mature mice was restricted to approximately 30% of the normal caloric requirement the average "true digestibility" of extracted whole egg was found to be 95.4 $\pm$ 0.2%.

The data indicate that metabolic fecal nitrogen values determined with protein-free or low protein diets are not safe indices of the metabolic fecal nitrogen under conditions of protein feeding.

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THE USE OF MICE FOR THE MEASUREMENT OF THE GROWTH PROMOTING QUALITY OF PROTEINS

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FIVE FIGURES

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Probably the most extensively used method for the determination of the nutritive quality of proteins for growth is the protein efficiency method of Osborne, Mendel and Ferry ('19). The nutritive quality of a protein is expressed as the ratio of body weight gain to protein consumed determined with growing rats that are fed the test protein under specified conditions. As commonly employed, this procedure has been subjected to numerous modifications by different workers. This lack of a standardized procedure makes it difficult to compare and evaluate the results from different laboratories.

The relative growth rate of the albino mouse is approximately twice that of the rat. If the protein efficiency method could be adapted to the mouse as the test animal it should be possible to decrease the time necessary for a growth measurement which, coupled with the smaller food requirements, would result in a saving of time, labor, and dietary ingredients.

The purpose of this study was to establish a rational procedure for the use of mice for protein efficiency determinations and to investigate the effect of such factors as pre-test standardization of test animals, the duration of the feeding period, and the level of the test protein in the diet.

EXPERIMENTAL METHOD

Male, weanling, albino mice (Sharp and Dohme, Swiss-Webster strain) were used in all studies. The mice were removed from the stock colony at the age of 15 to 16 days. Although the weight range at this age was 5.5 gm. to 9.5 gm., only those mice weighing from 7.0 gm. to 9.0 gm. were used. They were placed in individual wire bottom cages, and given water and the test diets ad libitum. All of the test periods were of 20 days' duration. To facilitate the recovery of feces and spilled food, with a minimum of urine contamination, blotting paper was placed below the cages. Daily records were kept of the animal weights and food consumption. All weighings were made to the closest 0.1 gm. The feces for each test group were pooled for the entire test period in 10% sulfuric acid. At the conclusion of each test period, the mice were killed with gas, and the carcasses saved for total body nitrogen determinations.

Preliminary experiments have shown that a fasting period of from 3 to 4 hours is sufficient for an almost complete emptying of the intestinal tracts of mice. Therefore, in order to eliminate the nitrogen in the intestinal contents in the determination of body nitrogen, the animals were killed 3 hours after the removal of the food from the cages at the last weighing. It was felt that this method would involve a smaller error than would a washing procedure.

Total nitrogen determinations of the diets, feces, and carcasses were made by the Kjeldahl method with modifications depending upon differences in sample sizes. For all determinations, the entire samples were partially digested and aliquot portions were used for the final digestion and distillation procedures.

The three protein sources used in these studies were acetone extracted, heat coagulated whole egg, casein,[1] and wheat gluten. They were incorporated into a basal ration which consisted of 25% hydrogenated cottonseed oil,[2] 2% corn oil,[3]

[1] Borden's "Labco" casein.
[2] Primex.
[3] Mazola.

20% glucose,[4] 4% salt mixture (Hubbell, Mendel and Wakeman, '37), 2% cellu flour, 1% Wilson's 1:20 liver concentrate powder, and sufficient white dextrin to make 100%. All diets were supplemented to contain per 100 gm.: 4 mg. of alpha tocopherol, 900 U.S.P. units of vitamin A, 180 U.S.P. units of vitamin D, 1 mg. of 2-methyl-l, 4-naphthoquinone diacetate, 0.8 mg. of thiamine hydrochloride, 1.6 mg. of riboflavin, 0.8 mg. of pyridoxine hydochloride, 4.0 mg. of niacin, 4.4 mg. of calcium pantothenate, 4.0 mg. of para-aminobenzoic acid, 200 mg. of choline chloride, and 21.6 mg. of inositol. This diet has been shown by Bosshardt et al. ('45b) to support good growth of mice.

The effect of the pre-test treatment of the animals

It has been observed that a change in the environment of the growing mouse causes a definite break in the growth curve. When weanling mice are transferred from the stock colony to the individual test cages they always show a loss of body weight the first day. The time required to regain this loss has been found to vary with the diet. When Purina Fox Chow or purified diets containing 10% or 20% casein were fed, the initial weight was regained after 4 days. When a purified diet containing 15% extracted whole egg was fed, the initial weight was regained in 2 to 3 days.

Four groups of seven male weanling mice were fed a test diet containing 10% casein for 20 days following each of the four pre-test treatments: (A) no holding period, (B) a 2-day pre-test period with a purified diet containing 15% extracted whole egg, (C) a 4-day pre-test period with Purina Fox Chow, and (D) a 4-day pre-test period with a purified diet containing 20% casein. The average growth curves and the protein efficiency ratios calculated at 10 days and at 20 days are shown in figure 1. Although the growth curves have the same slope, there is a marked difference between the protein efficiency ratios obtained after a preliminary 2-day feeding period

[4] Cerelose.

with a 15% extracted whole egg diet and those obtained after the other pre-test treatments. This difference is more pronounced for the values calculated at 10 days than for the values calculated at 20 days. These data indicate that growth rates are not reliable indices of protein utilization. The differences observed in the protein efficiency ratios indicate that

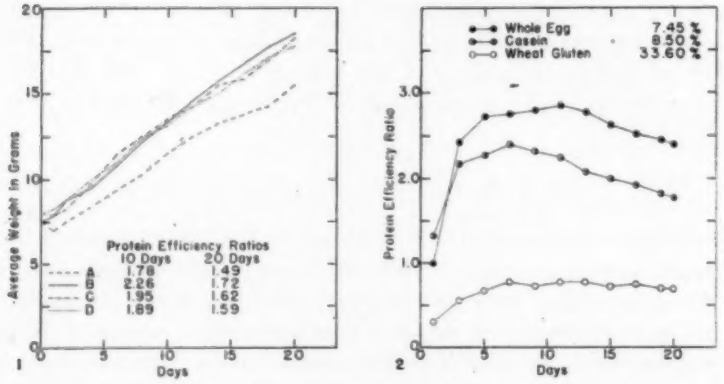

Fig. 1 The effect of pre-test treatment on the growth and protein efficiency ratios of mice receiving a purified diet containing 10% casein.

Fig. 2 The relation between time and protein efficiency ratios.

following a 4-day period of body weight loss and regeneration a greater protein intake is necessary to attain a definite growth rate than is required following the shorter 2-day period of body weight loss and regeneration.

As a result of these findings, in all subsequent studies a 2-day pre-test standardizing period with a purified diet containing 15% extracted whole egg was employed. This procedure was chosen because it was felt that, although all animals had gone through a short period of depletion and regeneration, any adverse effect would be minimized with the shorter period. As a further refinement, only those mice that had come to within 0.5 gm. or less of attaining their initial weight after the 2-day pre-test period were used.

The effect of the duration of the test period

The three protein sources, extracted whole egg, casein, and wheat gluten were incorporated into the basal ration to supply protein levels of 7.45%, 8.50%, and 33.5% (N × 6.25), respectively.[5] Twenty-day growth studies were made with groups of seven mice each that had been selected as previously described. The protein efficiency ratios were calculated daily throughout the experiment. The results are shown in figure 2. The optimal time for most constant results appears to be between 7 and 12 days since during this period there was a tendency for the slope of the curves to decrease. As the periods were continued beyond 12 days there was a gradual but definite decrease in the protein efficiency ratios of the better proteins. This was due to a slight decrease of the growth rate that occurred after 10 to 12 days with these diets. Since the food consumption, and thus the protein intake, did not decrease but gradually increased, the protein efficiency ratio decreased. Although all experiments in this report were of 20 days' duration, routine protein efficiency ratio determinations in our laboratories now are calculated at the end of 10 days.

The effect of the level of the test protein in the diet

When expressing the nutritive quality of a protein as its protein efficiency ratio, Osborne, Mendel, and Ferry specified the level of protein in the diet at which the maximal ratio was obtained. They suggested that this dietary level be established for each protein tested. In the most generally employed modifications of the method a single level of test protein, usually approximating 10%, is employed. As has been shown by Osborne, Mendel, and Ferry and by Barnes, Maack, Knights, and Burr ('45) the level of the test protein in the diet at which the maximal protein efficiency ratio is obtained varies with different proteins. Their data indicate that pro-

[5] These levels were selected from data presented in the following section as approximating those showing optimal protein efficiency ratios.

teins of poorer quality must be fed at relatively high levels to obtain the maximal protein efficiency ratios.

The three protein sources were incorporated into the basal ration, each at eight different levels ranging from 3% to 40%. Each diet was fed for 20 days to seven mice that had been selected as previously described. The protein efficiency ratios calculated at 10 days and at 20 days are shown in figures 3 and 4. The dietary level at which maximum utilization for growth was obtained was very definite for the better quality proteins,

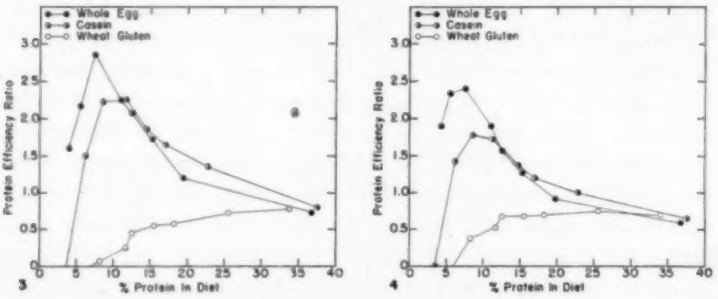

Fig. 3 The effect of the protein level in the diet on protein efficiency ratios at the end of a 10-day feeding experiment.

Fig. 4 The effect of the protein level in the diet on protein efficiency ratios at the end of a 20-day feeding experiment.

extracted whole egg and casein. This point was not so definite for the poorer quality protein, wheat gluten. These data tend to confirm the results mentioned earlier which were obtained with rats and which indicate that the poorer the protein the higher must be its level in the diet for maximal utilization for growth.

Although all growth studies included in this report are of 20 days' duration, the marked similarity existing between the curves calculated at 10 days and at 20 days, figures 3 and 4, indicates that valid results may be obtained by using a 10-day test period.

In expressing protein utilization for growth as the protein efficiency ratio, body weight gain is used as an index of body

protein gain. This gives rise to a criticism of the method. The protein content of the growing animal is not a constant factor. Weanling mice after the 2-day pre-test period have been found to contain $2.54 \pm 0.02\%$ N.[6] The body nitrogen levels and the average body weight and body nitrogen gains after the 20-day feeding periods are shown in table 1. It is evident that weight gain is not necessarily a true index of body nitrogen gain. The most striking example is that shown

TABLE 1

The body weight and body nitrogen gains and body nitrogen levels of mice receiving various diets for 20 days.

EXTRACTED WHOLE EGG				CASEIN				WHEAT GLUTEN			
Nitrogen in diet	Average body weight gain	Average body nitrogen gain	Nitrogen in carcasses	Nitrogen in diet	Average body weight gain	Average body nitrogen gain	Nitrogen in carcasses	Nitrogen in diet	Average body weight gain	Average body nitrogen gain	Nitrogen in carcasses
%	*gm.*	*mg.*	%	%	*gm.*	*mg.*	%	%	*gm.*	*mg.*	%
0.66	3.94	129	2.79	0.57	0.0	8	2.66	0.92	−0.13	12	2.75
0.87	7.01	217	2.80	1.00	4.1	137	2.72	1.30	1.11	43	2.72
1.19	11.22	339	2.82	1.36	9.0	286	2.88	1.87	2.20	74	2.74
1.73	12.90	396	2.86	1.78	12.6	357	2.74	2.00	3.49	110	2.74
2.02	12.68	397	2.91	2.35	12.3	379	2.86	2.48	4.41	144	2.82
2.44	12.28	412	3.05	2.71	12.9	432	3.05	2.93	5.56	180	2.85
3.14	10.89	452	3.42	3.63	13.6	435	2.96	4.08	9.41	288	2.83
5.86	12.93	402	2.90	6.00	13.5	428	2.94	5.37	10.41	336	2.94

by the three highest levels of whole egg protein. At dietary nitrogen levels of 2.44%, 3.14%, and 5.86% the average weight gains were 12.3 gm., 10.9 gm., and 12.9 gm., respectively. The average body nitrogen gains, however, were 412 mg., 452 mg., and 402 mg., respectively. The body nitrogen gains were determined as the difference between the level at the start of the experiment (weight $\times$ 2.54%) and the determined level at the conclusion of the experiment. Under the conditions of protein feeding at the level giving maximal utilization for

[6] Standard error $= \sqrt{\frac{\Sigma d^2}{n\,(n-1)}}$

growth the body nitrogen levels were essentially the same for the three proteins: extracted whole egg 2.82%, casein 2.88%, and wheat gluten 2.83%. Therefore, at dietary protein levels at which maximal protein efficiency ratios are obtained body weight gains may be used as indices of comparative body protein gains. However, as the body protein level changes during a growth study the use of body weight gains even at the dietary level showing maximum utilization for growth cannot be used as absolute indices of the utilization of ingested or absorbed protein for body protein gain.

A more valid expression of protein utilization for growth would be the utilization of ingested or, preferably, absorbed protein for body protein gain. Protein intake under conditions of ad libitum feeding may be varied by varying the protein level of the diet. The determination of body nitrogen gains at different levels of protein intake makes it possible to compare different proteins at equal intake levels using ad libitum feeding. It is also possible to determine the level of intake at which maximal utilization for growth is obtained. If "true digestibilities" are used, the calculations can be based on absorbed protein.

The "true digestibilities" of the three proteins were determined as described by Bosshardt and Barnes ('45a). The amount of fecal nitrogen per 100 gm. of food consumed was plotted against the nitrogen intake per 100 gm. of food at the different levels of protein intake. The calculated straight line was extropolated to the point of zero nitrogen intake to obtain the metabolic fecal nitrogen value under conditions of protein feeding. The average "true digestibilities" obtained were: extracted whole egg 92.5 $\pm$ 0.6%, casein 98.3 $\pm$ 0.1%, and wheat gluten 98.3 $\pm$ 0.03%.

The relationship between absorbed protein and the percentage of absorbed protein utilized for body protein gain for the three proteins during the 20-day test period is shown in figure 5. This method of calculation considers differences in food consumption, and thus in protein intake, that are omitted in figures 3 and 4. These data indicate that there is a

shift of the point of maximal utilization for growth to higher levels of protein absorption accompanying a decrease in the nutritive quality of the protein. It is, however, possible to compare the three proteins at a level of absorption approximating utilization for each. An approximate maximal utilization was observed when 5 gm. of each protein were absorbed per mouse for the 20-day period. This level of protein absorption would correspond to diets containing approximately 7.5% of extracted whole egg, 8.5% of casein and 12.5% of

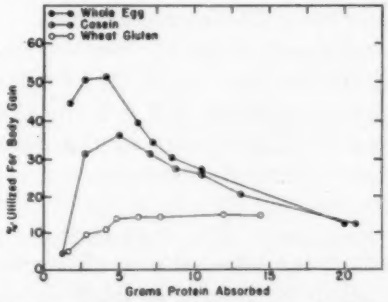

Fig. 5 The utilization of absorbed protein for body protein gain at different levels of protein intake.

wheat gluten. The true dietary levels for maximal utilization would be approximately 6.5% of extracted whole egg, 8.0% of casein, and 25% of wheat gluten corresponding to absorptions of 3.5 gm., 4.5 gm., and 12.0 gm., respectively.

DISCUSSION

The data presented in this report indicate that the albino mouse is a suitable test animal for the determination of the nutritive quality of proteins for growth. The advantages of using the mouse instead of the rat are several. A reliable growth result may be obtained in 10 days. Thus a marked saving of time is realized since the conventional test period for the rat method is 28 to 42 days. Approximately one-seventh the amount of test protein and other dietary ingredients are needed because of the saving in time and the smaller food

consumption of the mouse as compared with the rat. The saving in time and dietary ingredients realized by the use of the mouse will facilitate fractionation and chemical studies that are dependent on animal assays. The mouse likewise lends itself to studies involving expensive materials or those difficult to prepare such as isotopically labeled compounds. The reduced time necessary for a study usually makes it unnecessary to prepare more than one lot of diet, which is an aid in assuring constancy of the diet. For studies involving the whole carcass, the size of the mouse lends itself to simplified procedures for total carcass analysis.

The absolute efficiency ratios are not necessarily the same for both rats and mice, but the results of a large number of assays indicate that proteins and protein hydrolysates fall in the same order of classification by the two methods.

Weight changes and food consumption for the mouse are considerably less than for the rat, so that measurements must be made more accurately. The data indicate that there are certain factors that can affect the result in protein efficiency measurements when the mouse is used as the test animal. Similar effects may be noted when other animal species are employed. Factors such as pre-test standardization of the animals, the duration of the test period, and the level of the test protein in the diet can be and should be standardized if it is to be possible to compare and evaluate results from different laboratories. Differences in the reported numerical nutritive indices of proteins may be due, at least in part, to differences in experimental technique. Other factors possibly influencing protein utilization measurements that should be investigated are caloric intake, ratios of fat and carbohydrate in the test diets, nature of the mineral and vitamin supplementations, amount of undigestible material in the ration, and the strain of the animal species employed.

SUMMARY

The method of Osborne, Mendel and Ferry for the measurement of protein efficiency ratios (grams gain in body weight

per gram of protein consumed) has been adapted for use with mice.

The importance of establishing standardized conditions with regard to the pre-test treatment of the test animals, the duration of the test period, and the level of test protein in the diet has been demonstrated.

The advantages of the use of the mouse as compared with the rat are: smaller animal size, lower food consumption, and shorter test periods.

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ENERGY AND GASEOUS METABOLISM OF THE CHICKEN FROM HATCH TO MATURITY AS AFFECTED BY TEMPERATURE

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FOUR FIGURES

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INTRODUCTION

Metabolic activity is measured by energy output. Energy is required for work, for digestion and assimilation of feedstuffs and also to maintain body temperature. Since the energy expended to maintain body temperature is dependent on temperature of environment, this factor could have a profound influence on metabolic activity and thus on the feed consumption and meat and egg production of the chicken.

In a previous investigation, Barott and Pringle ('41) studied the effect of different environmental temperatures on the metabolism of the hen. In the investigation herein reported, the program of work was enlarged to include the life cycle of chickens from hatch to maturity, and a range of temperature from 20°F. to 103°F.

APPARATUS AND PROCEDURE

The data at temperature of 40°F. and above were obtained by use of one of the respiration calorimeters in the calorimetry laboratory of the Bureau of Animal Industry, Agricultural Research Center, Beltsville, Maryland. The details of construction of the calorimeter and technique of operation were

described by Barott ('37). It was necessary to alter the calorimeter for work at temperatures below 40°F. to a low temperature respiration chamber because the temperature control system could no longer function below this temperature. This alteration was accomplished by removing the heat absorber coil and inserting a cooling unit connected to a small refrigerating machine. The heating and cooling coils for the boundary control were also removed and 2 inches of cork insulation were added around the chamber. Therefore it was impossible to measure heat loss at temperatures below 40°F. It was also impossible to determine the water elimination because of the deposition of the moisture in the form of frost on the refrigeration unit. Accurate measurements of oxygen consumption and carbon dioxide elimination were obtained, however.

The procedure was similar to that with hens (Barott and Pringle, '41), except that the baby chicks and chicks 2 weeks of age were placed in a cage made of fine-meshed copper screen, divided into compartments. Each compartment was sufficiently large to accommodate one chick. While freedom of movement was not so severely restricted as in the case of the older birds no huddling was possible. Paper towels were put under the cages in the calorimeter to catch the down and any other material that came from the chicks and passed through the cages. This was done so that an accurate weight of the chicks could be determined. The weight of the feathers, droppings, etc., were subtracted from the beginning weight as noted under "Results."

The chickens 5 weeks, 8 weeks, and 12 weeks of age were put in individual galvanized wire cages. These cages were placed in the calorimeter over a pan of oil to catch the droppings. The chickens older than 12 weeks were placed in the calorimeter in individual cages similar in construction to those used for hens (Barott and Pringle, '41) except that they were smaller. These cages were also set over oil.

The chickens used were Rhode Island Red females. They were kept in battery brooders and fed the all-mash starting and growing diet no 2 (Titus, '41). The hens were fed a nor-

mal laying diet, and were housed in a partially open front laying house of the type used at this station (Mohler, '39).

The chickens were brought to the laboratory at 5 A.M., so that little, if any, feed was consumed since the previous night. They were weighed at the beginning and end of each experiment. During the time they were in the calorimeter they were in complete darkness and had no feed or water.

During the experimental period the air temperature within the calorimeter was kept practically constant at a pre-determined value, the relative humidity was kept between 50 and 60%, the oxygen content at 21% and the carbon dioxide below 1%.

A total of more than 500 1-day experiments were performed at approximately 5°F. intervals within a range of temperature from 20°F. to 103°F. The range limit for each age studied was determined by the survival of the chickens.

The oxygen consumption was measured for each 2-hour period and the carbon dioxide, water, and heat elimination for each 4-hour period.

The investigation was started with two groups of baby chicks, 2 to 6 days old, 100 in each group, hatched 1 week apart. No chickens from either group were used for more than 1 day's experiment at any one age. After the day's experiment they were put back into the brooder or on the range to grow until they reached the next age to be studied.

The ages investigated were decided upon after a study of the growth curve (fig. 2) which indicated that there might be greater changes occurring in the metabolic rate for the chickens up to 12 weeks of age than in that of the older ones. Therefore studies were made using baby chicks, and chickens 2 weeks, 5 weeks, 8 weeks, and 12 weeks old. As chickens begin laying at 4 to 6 months of age, the next age studied was 18 weeks, then 23 weeks, and finally 1 year. This range of age covers the growth period fairly well.

The data on oxygen consumption, and heat, water and carbon dioxide elimination obtained during the experimental period were computed, compiled and prepared for analysis for each

individual experiment. All volumetric values for oxygen consumption and carbon dioxide elimination were reduced to a common basis: 0°C. and 760 mm. atmospheric pressure.

In the analysis of the data the question arose as to what basis of comparison to adopt, i.e., whether to express the results as a function of body surface of the animal or of some power of the body weight. It is hard to determine with accuracy the body surface of a live animal, especially one covered with feathers. However, it is very easy to obtain an accurate weight. As metabolic activity depends upon the amount of active protoplasm in the living cells of the body, it is difficult for the authors to see wherein the body surface factor is any more accurate, if as accurate, as the body weight and their view is upheld by other investigators. Kleiber ('32) in an exhaustive treatise on the subject, states in his conclusions: "A power function of the body weight gives a better defined unit for measurement than the unit of body surface." Therefore, all results in this investigation are expressed as a function of the body weight and since there is no agreement among investigators as to what the power coefficient of the weight should be, we have expressed all results as a function of the weight to the first power. The initial weight of the bird, minus the weight of the excrement, was used in computing the initial value for energy and gaseous metabolism. The final weight was used in computing the final values. For computing the intermediate values, the difference between these two weights was pro-rated lineally with time.

RESULTS

The results of our previous work on hens (Barott and Pringle, '41) were shown by curves. The hens used in those experiments varied in weight from 1800 to 2800 gm.; however, in plotting the curves no account was taken of the variation in metabolism per gram due to this difference in the size of the hen. When the data on hens were analyzed with due regard for variation in weight, the double flexure previously shown in the curve for metabolic activity disappeared and the curve

assumed the form shown in this paper. The results previously published (Barott and Pringle, '41), when adjusted for variations in weight agree very well with those shown herein.

ENERGY AND GASEOUS METABOLISM

Values for oxygen consumption and for carbon dioxide and heat elimination were computed from observed data for each experiment. All the results for experiments at one temperature, with chickens of the same weight, were plotted on one chart and a curve drawn through the plotted points. This curve represented the metabolic activity of a chicken of given weight at one definite temperature. In figure 1, the oxygen consumption at 70°F. $\pm$ 2.0°F. of chickens of various ages is shown. The curves (fig. 1) all have the same general form for metabolic activity for the 24-hour period and are of the same type as those previously published. They all show the typical diurnal rhythm (first reported from this laboratory, Barott et al., '38) with a high value for metabolic rate at 8 A.M., gradually declining to a low at 8 P.M. and thereafter rising to another high at 8 A.M. the next day.

Normally, the value at 8 A.M. the second day would be of the same magnitude as that of the first day. However, because the chickens had no feed or water for the duration of the experiment, the second day's value is 5 to 10% lower except for baby chicks and for them the value the second day is the same as that for the first day because of the unabsorbed yolk upon which the chick feeds.

The magnitude of the metabolic activity when expressed per gram weight of chicken per hour is greatest for baby chicks and becomes progressively less and less as the chick gets older. It is least for the oldest chickens. However, the total metabolism (the value in units per hour per gram weight of chicken, times the weight of the chicken in grams), is least for the baby chicks and becomes greater very rapidly as the chicken's weight increases from 40 gm. to 300 gm. The rate of increase then becomes less and less as the age and weight

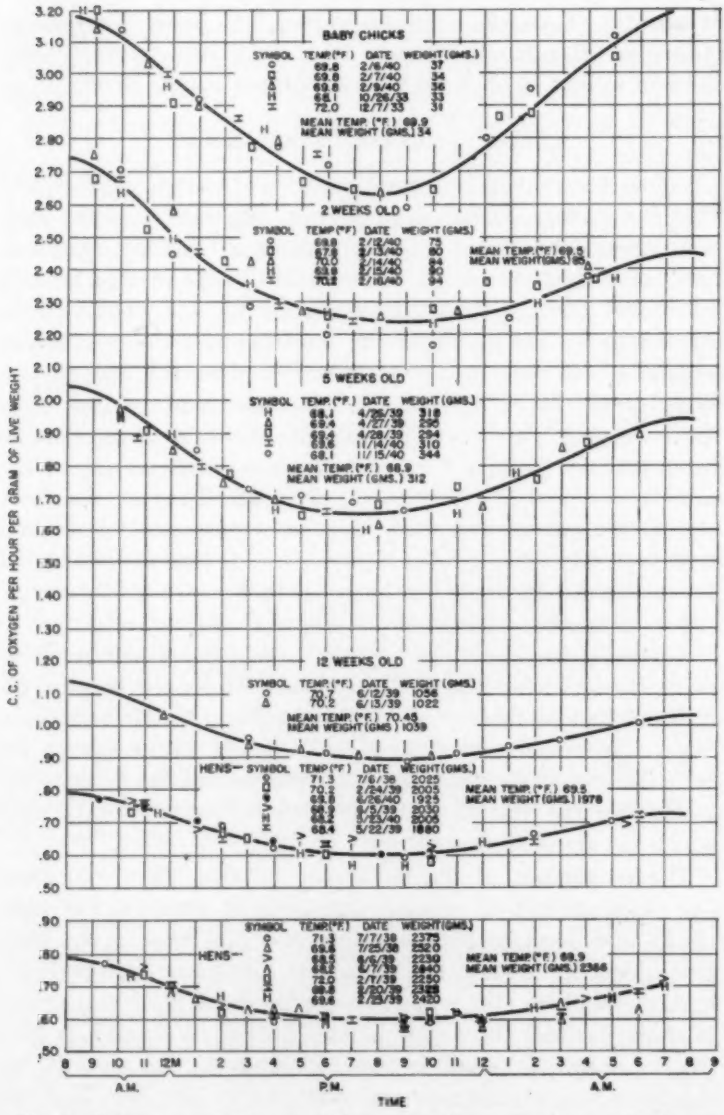

Fig. 1 Oxygen consumption of chickens of various ages at an environmental temperature of 70°F.

of the chicken increase, until it is practically zero at weights over 2800 gm.

By integration of curves similar to those in figure 1, the mean metabolic rate between 8 A.M. and 8 P.M. was determined for each experiment. The values thus obtained were plotted (fig. 2) and curves drawn through the plotted points in such a manner that they represent the mean value of the plotted points as closely as possible. These curves delineate the mean metabolic rate for each 10-degree interval from 30° to 90°F. Curves for 70°, 80°, and 90°F. delineate the metabolic rate for the life cycle of the chick from hatch to maturity.

Curves below 70°F. extend to the weight and age at which the chicken could survive the temperature studied. For example, baby chicks and chicks 2 weeks old did not survive 24 hours at temperatures below 70°F., those 5 weeks old did not survive below 50°F., etc. The lowest survival temperature for chickens older than 8 weeks has not yet been determined.

It will be noted that the trend of all curves is the same and that the metabolic rate decreases as the age and weight of the chicken increase. This decrease is very rapid during the first few weeks of growth, but becomes less and less until, between the weights of 2400 and 2800 gm., the rate is nearly constant.

Curves for carbon dioxide and heat elimination have the same form as those for oxygen consumption and differ only in absolute value. They have been omitted for the sake of brevity, as have those for oxygen consumption, and carbon dioxide and heat elimination for temperatures of 25°, 35°, 45°, 55°, 65°, 75°, and 85°F. However, all these curves were plotted and analyzed so that the curves shown in figure 3 could be constructed from the values.

Each curve in figure 3 shows the variation in metabolic rate (mean oxygen consumption between 8 A.M. and 8 P.M.) with temperature for a given weight of chicken. These curves (fig. 3) cover the life cycle of the chicken from the time of hatch to maturity. The values plotted on these curves were taken from figure 2 and similar curves. The carbon dioxide elimina-

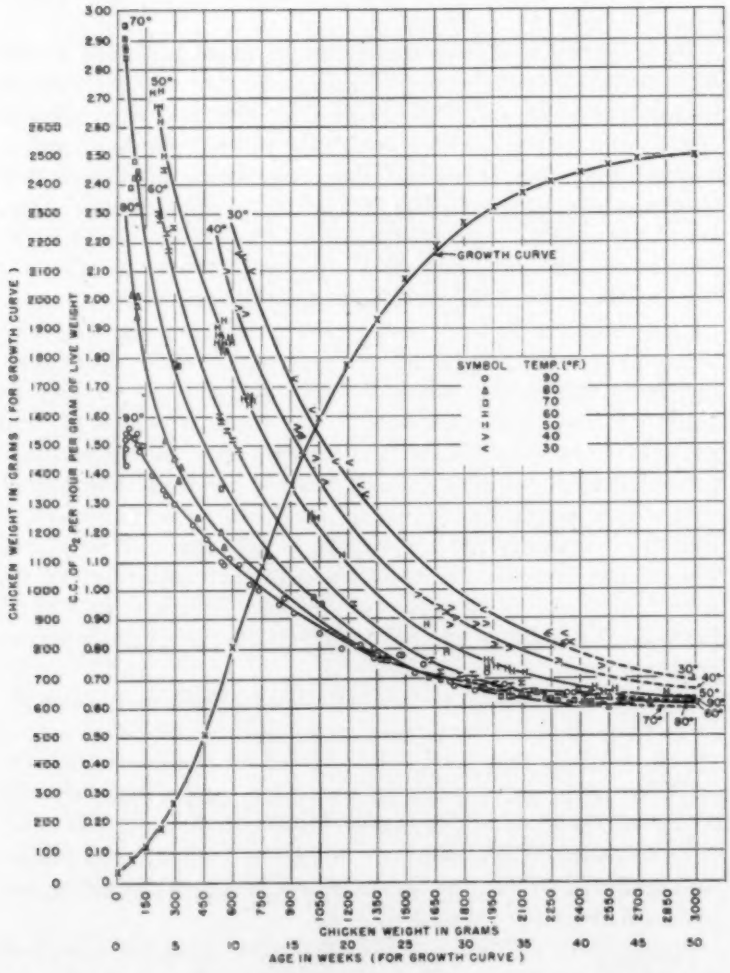

Fig. 2 Mean oxygen consumption from 8 A.M. to 8 P.M. of chickens from hatch to maturity at various environmental temperatures and growth curve. (Source of data for growth Curve: research by poultry section. A. H. Div., B. A. I., Agric. Res. Center, Beltsville, Md.)

tion and the heat elimination for the same groups of chickens as those in figure 3 were determined in the same manner as the oxygen consumption. However, as the type of curve is the same, the curves are not shown but the values are tabulated in tables 1 and 2.

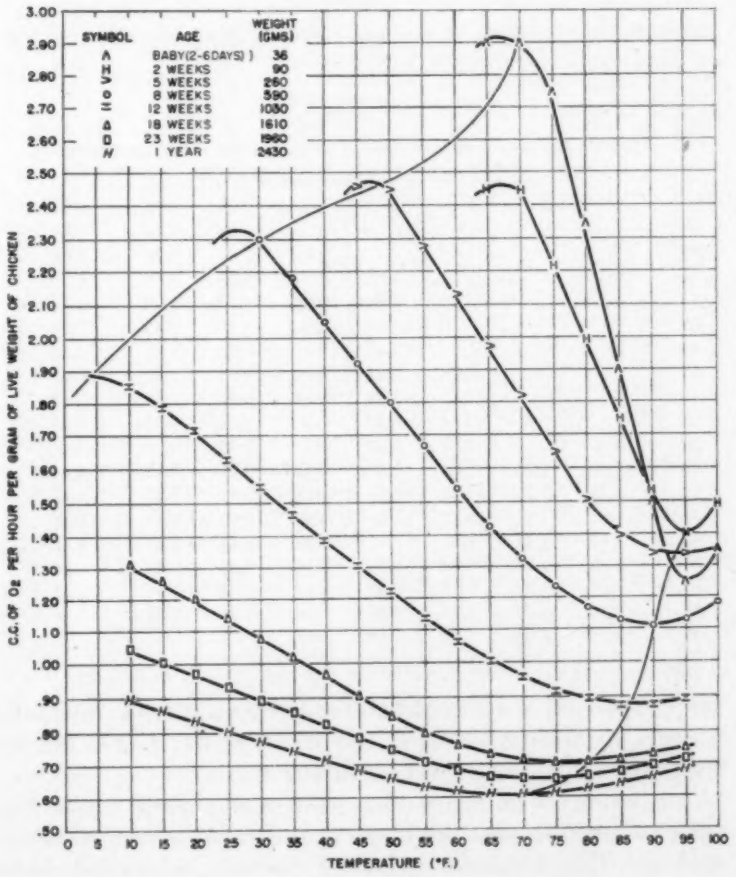

Fig. 3 Oxygen consumption of chickens at various temperatures: mean of 8 A.M. to 8 P.M. values.

The upper temperature limits for survival for 24 hours under the conditions of these experiments lie between 90°F. for mature fowls and 103°F. for baby chicks. The temperature limits become progressively lower than 103°F. as the age of the chick increases. Some hens died during experiments at

TABLE 1

Carbon dioxide elimination of chickens at various environmental temperatures. Mean values between 8 a.m. and 8 p.m.

TEMPERATURE	CHICKENS							
	Age	0-1	2	5	8	12	18	23	52 weeks
	Wt.	36	90	260	590	1030	1610	1960	2430 gm.
°F.	*All values are ml. CO_2 per hour per gram live weight*								
10						1.34	0.94	0.76	0.64
15						1.28	0.89	0.73	0.62
20						1.22	0.86	0.70	0.60
25					1.67	1.16	0.81	0.68	0.58
30					1.65	1.10	0.77	0.65	0.56
35					1.56	1.04	0.73	0.62	0.54
40					1.47	0.99	0.69	0.59	0.51
45				1.78	1.38	0.93	0.65	0.57	0.49
50				1.75	1.30	0.88	0.61	0.54	0.48
55				1.63	1.20	0.82	0.57	0.51	0.46
60				1.52	1.10	0.77	0.54	0.49	0.45
65		2.08	1.75	1.41	1.02	0.72	0.52	0.48	0.44
70		2.08	1.75	1.30	0.94	0.69	0.51	0.47	0.44
75		1.97	1.58	1.25	0.88	0.66	0.51	0.47	0.44
80		1.67	1.42	1.08	0.83	0.64	0.51	0.48	0.45
85		1.35	1.26	0.99	0.80	0.64	0.52	0.48	0.46
90		1.07	1.10	0.96	0.80	0.63	0.53	0.49	0.47
95		0.90	1.00	0.96	0.82	0.64	0.54	0.51	0.49
100		1.00	1.08	0.99	0.86	0.66			

90°F., chickens 8 weeks old and older died at 95°F., chickens 2 weeks and 5 weeks old, survived at 100°F., but died at 102°F., while the baby chicks survived at 103°F.

All the curves on figure 3 have the same general form. The metabolic rate is at a minimum at a definite temperature for each age. This temperature is 95°F. for baby chicks and chicks 2 weeks old and as the chicken gets older the temperature at which metabolism is at a minimum is progressively lower,

until for 1-year-old chickens the minimum metabolism occurs at approximately 70°F.

The metabolic rate increases as the temperature is decreased below that at which the minimum metabolism occurs and continues to increase until the chicks are no longer able to produce enough heat to maintain body temperature and perish from the cold. The metabolic rate increases most rapidly for the baby chicks and becomes progressively less and less as the chicken gets older. The rate also increases at temperatures

TABLE 2

Heat elimination of chickens at various environmental temperatures. Mean values between 8 a.m. and 8 p.m.

TEMPERATURE	CHICKENS							
Age	0-1	2	5	8	12	18	23	52 weeks
Wt.	36	90	260	590	1030	1610	1960	2430 gm.
°F.	*All values are calories per hour per gram live weight*							
40				9.10	6.15	4.25	3.65	3.20
45			11.00	8.55	5.80	4.00	3.50	3.10
50			10.80	8.00	5.45	3.75	3.35	2.95
55			10.30	7.45	5.10	3.55	3.20	2.85
60			9.60	6.80	4.75	3.35	3.10	2.80
65	12.90	10.85	8.90	6.30	4.50	3.25	3.05	2.75
70	12.90	10.85	8.15	5.85	4.25	3.15	3.00	2.75
75	12.00	9.90	7.45	5.45	4.10	3.10	2.95	2.75
80	10.30	8.85	6.70	5.15	4.00	3.10	2.85	2.80
85	8.40	7.85	6.20	5.00	3.90	3.15	3.00	2.85
90	6.60	6.80	5.95	4.95	3.90	3.25	3.10	2.90
95	5.50	6.25	5.95	5.00	3.95	3.40	3.20	3.00
100	6.00	6.50	6.20					

above that for the minimum metabolic rate. This increase is due primarily to the extra effort entailed in panting to evaporate sufficient water from the respiratory tract for cooling purposes in maintenance of body temperature. The increase in rate continues to a point where the bird can no longer evaporate sufficient water for cooling and perishes from the heat. The great increase in water elimination for the ages studied is shown in figure 4. The flexures at the low temperature end of the curves for baby chicks, and chickens 2 weeks,

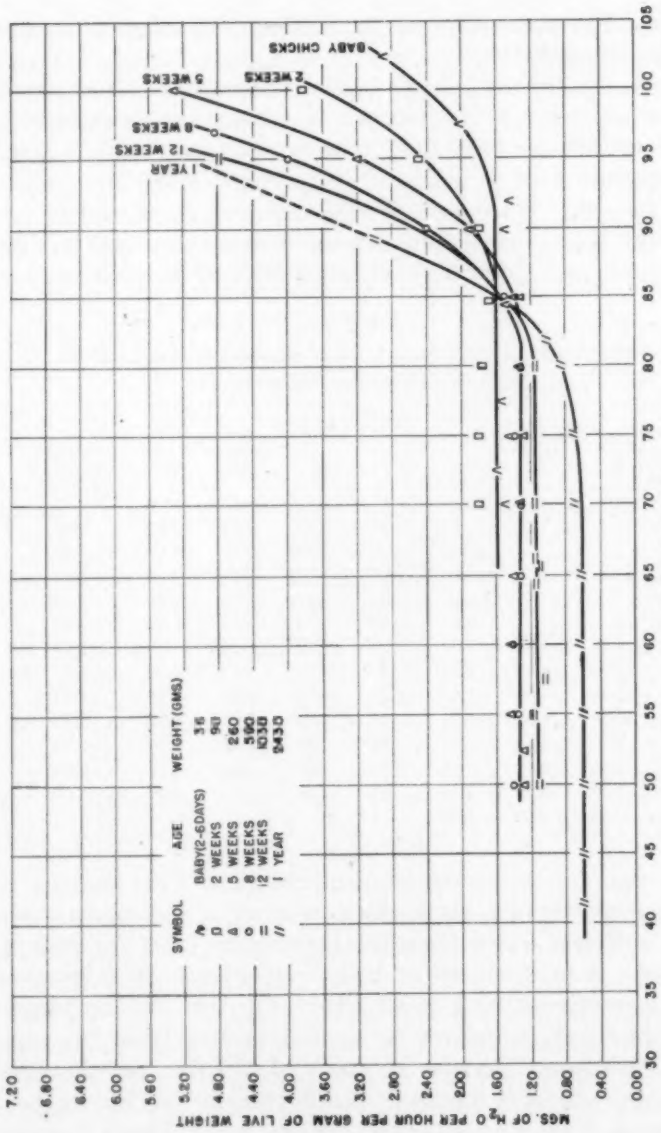

Fig. 4 Water of respiration.

5 weeks and 8 weeks old indicate the lowest temperatures that the chickens could survive for 24 hours without ill effects, under the conditions of the experiment.

The last point plotted on each of these four curves (at 65°F. for baby chicks and chickens 2 weeks old, 45°F. for 5-week-old and 25°F. for 8-week-old chickens) was obtained from results for short intervals of time as the chickens could not stand 24 hours' exposure. The lowest temperature at which chickens older than 8 weeks can survive under similar conditions has not yet been determined. It will be noted that chickens 2 weeks of age were unable to survive a temperature lower than the limit for baby chicks, i.e., 70°F.

Respiratory and thermal quotients and food material metabolized

The respiratory quotient and the carbon dioxide and oxygen thermal quotients were computed from observed data for the period from 8 A.M. to 8 P.M. There appeared to be very little variation in the respiratory or thermal quotients with either temperature or age of bird. All values of the R.Q. approximated 0.717. The R.Q. did seem to decrease slightly below 40°F., reaching a value of 0.715 at 10°F. Whether this is true or an artifact, it is impossible from the data to state. The oxygen T.Q. was 3.11 and the carbon dioxide T.Q. 3.16.

Equations [1] were set up in a previous paper (Barott et al., '38) for the computation of the relative quantities of carbohydrate, fat and protein being metabolized. Inserting the computed values of the respiratory and thermal quotients in these equations, we found that the relative amounts of the

[1] Inasmuch as direct measurements of both heat and gaseous metabolism were made, the relative quantities of carbohydrate, fat and protein being metabolized could be estimated from the R.Q. and the T.Q.'s. The method of estimation was as follows: If x, y and z represent, respectively, the percentages of carbohydrate, fat and protein being metabolized, the following equations may be set and readily solved: $1.000\ x + 0.707\ y + 0.705\ z = 100 \times$ the observed R.Q.; $2.568\ x + 3.373\ y + 3.110\ z = 100 \times$ the observed CO_2 thermal quotient; $3.531\ x + 3.279\ y + 3.018\ z = 100 \times$ the observed O_2 thermal quotient.

three food materials metabolized by the chickens in these experiments were: protein, 69%; fat, 27%; and carbohydrate, 4%.

Respiratory water elimination

Some difficulty was encountered in determining the amount of water eliminated by respiration for all chickens 12 weeks old and younger. The fine wire mesh of the cages necessary for baby chicks and those 2 weeks of age prevented the droppings from passing through and although the cages for chickens 5 weeks, 8 weeks, and 12 weeks of age were placed over a pan of oil in which the droppings were collected, some droppings always adhered to the cages. During the course of the experiment, the water in these exposed droppings evaporated and was absorbed with the water of respiration, making it impossible to get a correct figure for the latter. Determinations have shown, however, that the amount of water in the feces is approximately 75% of the total weight. Some tests made at this laboratory showed that the feces contained about 30% of water at the time of removal from the cages. Assuming these values to be correct, one can compute the approximate amount of water evaporated from the feces. Subtracting this amount of water from the total water collected in the absorption flasks, gave an estimate of the water eliminated by respiration.

The results were plotted in figure 4 and curves drawn through the plotted points. The points on the curve for the water of respiration of hens are observed values as the feces were collected under oil and did not introduce an error into the water collected in the flasks. The curves for the younger chickens show the general trend of the respiratory water elimination and although the magnitude of the error is not great, they are not to be considered as being of the same accuracy as the curve for hens.

No value for water elimination could be obtained below 40°F., because the precipitation of moisture on the cooling unit.

The water of respiration for each age studied remains fairly constant at all temperatures up to the temperature of minimum metabolism. Above this temperature it becomes necessary for the chicken to exhale an additional amount of water for the purpose of cooling. This amount rapidly becomes greater and greater as the temperature is increased. The water respired per gram weight of chicken, at temperatures below the minimum, is lowest for hens and becomes higher for the younger chickens. As previously stated, the data for chickens 12 weeks old and younger are only fairly accurate estimates and not true measurements as in the case of the hens.

SUMMARY AND CONCLUSIONS

The energy and gaseous metabolism of Rhode Island Red female chickens, averaging 4 days, 2 weeks, 5 weeks, 8 weeks, 12 weeks, 18 weeks, 23 weeks and 1 year old, were determined by use of one of the respiration calorimeters in the calorimetry laboratory of the Bureau of Animal Industry, Agricultural Research Center, Beltsville, Maryland. The instrument is similar to the one described by Barott ('37). More than 500 1-day experiments were made.

Results were obtained with environmental temperatures at approximately 5°F. intervals in the range from 20° to 103°F. Conditions other than temperature were: Relative humidity 50–60%, oxygen content 21%, and carbon dioxide content less than 1%.

The oxygen consumption was measured for each 2-hour period, and the heat, water and carbon dioxide elimination for each 4-hour period. The results define the metabolic rate of each age of chicken studied, at each environmental temperature investigated. A point of flexure occurs at the temperature where metabolism is a minimum. This minimum occurs at 95°F. for baby chicks and chickens 2 weeks old. As the chicken gets older the minimum occurs at a temperature which is progressively lower until for 1-year-old hens, the minimum occurs at approximately 70°F. The maximum meta-

bolism occurs at the lowest temperature the chicken could survive. This temperature has not yet been determined for chickens older than 8 weeks.

The data obtained for each 24-hour period show the typical diurnal rhythm in the metabolism of the chicken, with a maximum value at 8 A.M. and a minimum value at 8 P. M.

The amounts of protein, fat and carbohydrate metabolized by the chickens were computed. The amounts of the three food constituents metabolized were: protein, 69%; fat, 27% and carbohydrate, 4%.

The observed R.Q. approximates 0.717 at all ages and all temperatures. The observed oxygen T.Q. is 3.11 and the observed carbon dioxide T.Q. is 3.16.

The water of respiration is fairly constant at temperatures below that where minimum metabolism occurs. Above this temperature there is an enormous increase due to the large amount of water exhaled for cooling at the higher temperatures.

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THE THIAMINE, RIBOFLAVIN, NICOTINIC ACID AND PANTOTHENIC ACID CONTENT OF COLOSTRUM AND MILK OF THE COW AND EWE

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In most mammalian species the new-born under natural conditions depends for varying periods of time almost entirely on the secretion from the mammary gland for food. Colostrum differs in composition from milk in several respects. While extensive studies have been made of the carotene and vitamin A content of colostrum of the cow (Dann, '33; Henry, Houston and Kon, '40), sheep (Underwood and Curnow, '44) and human (Dann, '36) information on the B vitamins in colostrum is meagre. The report by Lundquist and Phillips ('43) on the possible role of some of the vitamins in the prevention of certain diseases of the new-born calf prompted the authors to make a study of the amounts of thiamine, riboflavin, nicotinic acid and pantothenic acid in colostrum as compared with milk. Information on the B vitamins in colostrum affords a basis for the preparation of substitutes in feeding new-born animals, and especially for the first few days of life which is a critical period.

The amount of thiamine in human milk is definitely influenced by the dietary intake (Knott et al., '43) and a similar relationship probably holds for the other B vitamins in colostrum and milk of species for which they are a dietary

essential. It was felt that this variable could be largely eliminated or reduced to a minimum if the studies were made with ruminants as the B vitamins are not dietary essentials for them.

EXPERIMENTAL

Methods

Samples of colostrum were obtained from cows on the day of parturition. Preliminary studies made on colostrum collected at frequent intervals after parturition showed that there was no essential change in any of the four vitamins under study during the first 24 hours. A sample of milk was collected from each of the cows after they had been in milk for 30 days or longer. The two breeds represented are the Holstein and Jersey.

The colostrum was collected from the ewes on the day of parturition. The samples of milk were not from the same ewes as the colostrum, but it is presumed that they would be representative for the species.

Precautions were taken to protect the samples against photochemical destruction of riboflavin. Thiamine was determined by the thiochrome method of Hennessy ('42). Riboflavin was determined by the microbiological method of Snell and Strong ('39) on the autoclaved sample following filtration at a pH of approximately 4.6 to remove foreign growth stimulants. Nicotinic acid was determined by the microbiological method of Krehl, Strong and Elvehjem ('43) with the slight modification devised by Pearson and Luecke ('45) which was found to be satisfactory for colostrum and milk. Pantothenic acid was determined by the method of Neal and Strong ('43) following the enzymatic liberation of the vitamin by takadiastase and papain.

RESULTS

The mean values and standard deviations for thiamine, riboflavin, nicotinic acid and pantothenic acid for colostrum and

milk of the cow and ewe are shown in table 1. There is a distinct species difference in the amount of riboflavin, thiamine and nicotinic acid in both the colostrum and milk. The colostrum of the ewe is approximately 74% higher in thiamine than the colostrum of the cow while for milk the corresponding difference is around 58%. The difference for riboflavin is more striking as the amount in the colostrum of the ewe is about 3.3 fold greater than in the colostrum of the cow while the corresponding figure for milk is about 2.5 fold. There is a similar relationship for nicotinic acid which is higher in

TABLE 1

Thiamine, riboflavin, nicotinic acid and pantothenic acid content of colostrum and milk.
(Values in μg. per ml.)

VITAMIN	COW					EWE				
	No. of animals	Colostrum		Milk		No. of animals	Colostrum		Milk	
		Mean	*S. D.*[1]	*Mean*	*S. D.*[1]		*Mean*	*S. D.*[1]	*Mean*	*S. D.*[1]
Thiamine	25	0.62	0.06	0.38	0.05	5	1.08	0.31	0.60	0.12[2]
Riboflavin	32	6.10	1.85	1.77	0.31	14	20.08	5.46	4.36	0.66
Nicotinic acid	32	0.96	0.29	0.91	0.16	14	1.97	0.44	3.93	1.09
Pantothenic acid	32	2.24	0.87	3.67	0.57	14	2.62	0.71	3.66	0.79

[1] Standard deviation.
[2] Twelve samples of milk.

both the colostrum and milk of the ewe than in cow's colostrum and milk. There is an interesting relationship between the rates of gain of the new-born and the amount of thiamine, riboflavin and nicotinic acid in the milk of the two species. The new-born lamb doubles its weight in approximately 14 days while the calf requires around 47 days to double its weight. One can postulate that the higher amounts of thiamine, riboflavin, and nicotinic acid in the colostrum and milk of the ewe constitute one of the functions of the more rapid growth of the new-born of this species. A limited number of observations in this laboratory has revealed that the amounts of these three vitamins in the milk of the human and horse is much lower than in cow's milk. The rate of growth of the

new-born of these species is likewise much slower than that of the new-born calf.

There are definite differences in the amounts of the vitamins in the colostrum and milk of both species. The difference between the mean values for milk and for colostrum has been tested by Fisher's t value. The difference in the values of colostrum and milk for riboflavin and pantothenic acid is highly significant for both species. The difference between the amount of thiamine in the colostrum and milk of the cow is highly significant, and for the ewe is significant. The difference in the amount of nicotinic acid in the colostrum and milk of the ewe is highly significant, but for the cow there was no significant difference.

The colostrum of the cow contained an average of 0.62 μg. of thiamine per milliliter while the milk contained 0.38 μg. per milliliter. The corresponding values for the colostrum and milk of the ewe are 1.08 μg. and 0.60 μg. per milliliter, respectively. The higher thiamine content of colostrum of both the cow and ewe differ from the findings of Knott et al. ('43) on human colostrum and milk. These workers reported that human colostrum contained almost no thiamine and that there was a gradual increase in the amount up to 3 weeks after parturition. In contradistinction to this Escudero and Sola ('43) reported that human colostrum contained 43% more thiamine than human milk. A possible explanation of these conflicting reports is that the pre-parturient dietary regimen of the subjects may have furnished quite different amounts of thiamine.

The colostrum of the cow contained an average of 6.10 μg. of riboflavin per milliliter while milk contained 1.77 μg. per milliliter which is a ratio of about 3.4 to 1. The values for riboflavin in the colostrum and milk of the ewe are 20.08 μg. and 4.36 μg. per milliliter, respectively. The higher riboflavin content of colostrum in these species is in accord with the higher value observed in human colostrum as compared with human milk by Escudero and Sola ('43).

The nicotinic acid content of colostrum and milk of the cow is not significantly different. However, the milk of the ewe

contains almost twice as much nicotinic acid as the colostrum. This is the only respect in which the general relationship between the amount of each vitamin in the colostrum and milk of the cow differs from that in the colostrum and milk of the ewe.

The milk of both the cow and ewe contains significantly more pantothenic acid than the colostrum of the respective species. The higher pantothenic acid content of milk and the higher nicotinic acid content of the milk of the ewe is in contrast with

TABLE 2

Effect of progress of lactation period on level of thiamine, riboflavin and pantothenic acid in cow's colostrum and milk.

DAYS AFTER PARTURITION	THIAMINE	RIBOFLAVIN	PANTOTHENIC ACID
	μg./ml.	*μg./ml.*	*μg./ml.*
0	0.58	5.69	1.73
1	0.59	3.53	3.20
2	0.59	2.67	3.96
3	0.59	2.32	4.24
4	0.58	2.03	4.01
5	0.59	2.03	4.05
6	0.58	1.93	4.19
7	0.57	1.87	4.29
8	0.56	1.87	4.38
9	0.56	1.89	4.16
30	0.38	1.83	3.82

thiamine and riboflavin which are higher in colostrum. The values reported here for the thiamine, riboflavin, nicotinic acid and pantothenic acid in cow's milk agree fairly well with the figures in the literature. They were included in the present study as they afford a more accurate control based on colostrum and milk samples from the same animals.

The daily change in the amount of thiamine, riboflavin and pantothenic acid in colostrum of the cow following parturition is shown in table 2. The data for nicotinic acid have been omitted from this table as there was no significant difference in the amount in cow's colostrum and milk. Samples were

collected the day of parturition and daily thereafter for 9 successive days. A sample of milk was collected again about the thirtieth day. The data in table 2 are average values for eight animals.

The average thiamine value of the colostrum for the eight cows the day of parturition was 0.58 μg. per milliliter and 9 days later it was essentially the same being 0.56 μg. per milliliter. The average thiamine content of the milk of the eight cows 30 days after parturition was 0.38 μg. per milliliter which is the same as that shown for milk in table 1. It is interesting that the high thiamine content of colostrum and subsequently of the milk persists for a much longer period than does the high riboflavin level. The riboflavin declines rapidly after the first day and approaches the normal value for milk by about the sixth or seventh day of lactation.

There is a marked increment in the pantothenic acid content of the colostrum as evidenced by the change from 1.73 μg. per milliliter the day of parturition to 3.20 μg. per milliliter on the second day. The pantothenic acid level approaches the normal level for milk about the third day following parturition. No particular significance is attached to the slightly higher levels observed a few days later.

SUMMARY

There is a definite species difference in the amounts of thiamine, riboflavin and nicotinic acid in both the colostrum and milk of the cow and ewe. The colostrum and milk of the ewe are both much richer in each of these vitamins than the colostrum and milk of the cow.

The colostrum of both the cow and ewe is much higher in thiamine and riboflavin than is the milk of the respective species. The colostrum of the cow contains an average of 0.62 μg. of thiamine and 6.10 μg. of riboflavin per milliliter while the corresponding values for the milk are 0.38 μg. and 1.77 μg. per milliliter. Ewe's colostrum contains 1.08 μg. of thiamine and 20.08 μg. of riboflavin per milliliter while the corresponding values for the milk of the ewe are 0.60 μg. and 4.36 μg. per

milliliter. There was no significant difference in the nicotinic acid content of cow's colostrum and milk, but ewe's milk contained almost twice as much as the colostrum. The pantothenic acid level of the milk of both species is higher than it is in the colostrum.

The riboflavin and pantothenic acid for the cow attain the level for normal milk within less than a week after parturition, but the high level for thiamine persists beyond the tenth day.

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VEGETABLE CROPS IN RELATION TO SOIL FERTILITY

IV. NUTRITIONAL VALUES OF NEW ZEALAND SPINACH [1]

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There is still a paucity of information on the question as to just how much green leafy vegetables contribute to human nutrition. Recently, they have been termed "protective" foods because of their dietary importance as sources of calcium and vitamins A, C, and G, which are among the nutrients most apt to be deficient in American dietaries (Report of the Committee on Diagnosis and Pathology of Nutritional Deficiencies Food and Nutrition, '43). However, with respect to calcium, members of the Goosefoot family of greens (spinach, Swiss chard, beet greens) and New Zealand spinach may contain sufficient oxalic acid to entirely nullify this nutrient contribution, as well as to render unavailable in the diet additional quantities of calcium in other foods such as milk (Kohman, '34, '39; Fincke and Sherman, '35; Fairbanks and Mitchell, '38; Tisdall and Drake, '38; Spiers, '39).

According to one report (Kohman, '34), New Zealand spinach contained an unusually high percentage of oxalic acid, its concentration being about equal to the toxic amounts in rhubarb leaves. Also, its vitamin C content has been listed at a relatively low figure (Hewston and Marsh, '42). Since this crop

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is so universally recommended as a desirable vegetable and as a good substitute for some other greens which cannot be grown so successfully during hot weather, additional information on its nutritional values, as compared with other leafy vegetables, was felt essential. Moreover, the effects of the soil and its fertility level on the nutrient quality of a given crop may alter its "protective" value, and are, therefore, commanding an increased attention in experimental studies with vegetables.

EXPERIMENTAL

New Zealand spinach was grown under controlled greenhouse conditions in gallon glazed crocks, using colloidal clay cultures as a soil medium (Albrecht and Schroeder, '39). A series of sixteen treatments was prepared by supplying calcium and nitrogen at levels of 5, 10, 20, and 40 milliequivalents (m.e.) with all possible (i.e. 16) combinations of these amounts of the two nutrients. Thus, for each level of calcium, there were four levels of nitrogen, and for each level of nitrogen, there were four of calcium. Each of the sixteen series was replicated ten times with two plants in each replication.

The calcium and nitrogen were provided by adding variable quantities of calcium acetate and ammonium nitrate, respectively, to the correspondingly required clay aliquots, replacing the naturally adsorbed hydrogen thereon with cationic nutrients. Other materials supplied, but in constant amounts for all treatments, consisted of 20 m.e. each of potassium and phosphorus and 6 m.e. of magnesium and sulfate.

The plants were grown for a period of about 70 days and the tops harvested, at which time they were judged to be in the most desirable eating condition. One specimen from each replication, making a total of ten individual plants separately analyzed in each treatment, was utilized for vitamin C analyses, conducted according to methods described by Wittwer, Schroeder and Albrecht ('45). The remaining ten plants from each treatment were harvested, dried, ground, and used collectively for the chemical determinations of oxalate, total

nitrogen, phosphorus, calcium, and magnesium. The A.O.A.C. ('40) methods for calcium and magnesium were used. Oxalate was measured according to Pucher, Wakeman and Vickery ('41), total nitrogen according to Murneek and Heinze ('37) and phosphorus according to Fiske and SubbaRow ('25).

RESULTS

The total growth of the plants and their composition, as related to increments of calcium and nitrogen, are given in table 1. The treatments are arranged into four nitrogen groups, in which the different calcium levels are averaged to show the influence of variable nitrogen. Regrouping, according to the calcium applied, is possible if the rather insignificant effects of calcium are desired.

The first outstanding feature observed with reference to the crop's behavior was its very decided response to nitrogen as a fertilizer salt. This influence manifested itself in both the quantity and quality of vegetation produced. Yields were a direct function (treatment 5 a possible exception) of the supply of nitrogen, almost without regard to the soil calcium supply. Within the edible portions of the plant, the vitamin C content was significantly depressed, as a result of increased nitrogen fertilization, which is in accord with recent reports on other fruits and vegetables (Jones et al., '44; Hamner, Bernstein and Maynard, '45; Wittwer, Schroeder and Albrecht, '45). Also, as more nitrogen was applied, decreases similar to those for vitamin C occurred in the concentrations of oxalate and phosphorus, though, as might be expected, the plants' total nitrogen percentages increased.

Of perhaps even more importance in human nutrition is the fact that New Zealand spinach is a very lean source for some of the essential food items, normally thought to be plentifully supplied in the diet by all green leafy vegetables. The concentration range for vitamin C, in milligrams per 100 gm. of fresh weight, was 20.9 ± 0.48 for high nitrogen plants to 29.6 ± 0.44 for those more deficient in this element (table 1). These values, when listed with those for other com-

TABLE 1

Variations in yield, vitamin C, oxalate, and minerals of New Zealand spinach grown under variable levels of nitrogen and calcium.[1]

TREATMENT	APPLIED		YIELD PER 10 PLANTS		VITAMIN C per 100 gm.	OXALATE	MINERAL ANALYSES			
	N	Ca	Fresh wt.	Dry wt.			N	PO_4	Ca	Mg
	m.e.	m.e.	gm.	gm.	mgm.	%	%	%	%	%
					N group I					
1	40	40	467.6	27.21	20.3 ± 0.70	6.07	6.12	1.63	0.460	0.554
5	40	20	280.0	16.58	18.9 ± 0.98	5.80	5.97	1.65	0.659	0.164
9	40	10	461.2	28.92	21.4 ± 1.01	6.05	6.07	1.73	0.456	0.262
13	40	5	466.2	29.88	22.8 ± 0.66	6.17	6.18	1.67	0.548	0.041
Average			418.8	25.65	20.9 ± 0.48	6.02	6.09	1.67	0.531	0.255
					N group II					
2	20	40	422.2	25.80	28.5 ± 0.87	7.08	4.79	2.34	0.536	0.393
6	20	20	367.7	22.17	27.9 ± 1.18	7.42	5.25	2.21	0.479	0.086
10	20	10	325.1	20.48	25.3 ± 1.54	7.13	5.60	1.78	0.602	0.337
14	20	5	392.1	24.90	26.9 ± 1.06	6.86	5.76	2.06	0.528	0.025
Average			376.8	23.34	27.2 ± 0.59	7.12	5.35	2.10	0.536	0.211
					N group III					
3	10	40	267.7	17.37	29.5 ± 0.63	7.95	3.58	3.68	0.570	0.343
7	10	20	230.8	14.73	28.7 ± 0.74	7.52	4.64	2.97	0.548	0.069
11	10	10	219.4	14.83	29.5 ± 0.57	7.24	4.76	2.52	0.520	0.389
15	10	5	158.5	11.29	30.5 ± 1.38	6.77	5.06	3.25	0.506	0.074
Average			219.1	14.56	29.6 ± 0.44	7.37	4.51	3.11	0.536	0.219
					N group IV					
4	5	40	142.2	9.40	27.0 ± 0.72	9.36	3.06	3.63	0.618	0.368
8	5	20	122.8	8.42	28.4 ± 1.02	7.34	3.33	4.00	0.634	0.340
12	5	10	121.5	8.43	30.0 ± 1.16	8.57	3.84	3.56	0.583	0.131
16	5	5	98.8	7.24	29.6 ± 0.80	7.42	3.82	4.40	0.514	0.324
Average			121.3	8.37	28.7 ± 0.49	8.17	3.51	3.90	0.587	0.291

[1] Vitamin C given in milligrams per 100 gm. fresh weight, oxalate and mineral analyses in percentage of dry weight.

mon greens, are very low. Members of the Goosefoot family (spinach, Swiss chard, beet greens) have a range of concentrations from about 40 to 100 mg., while for vegetables of the Mustard family (kale, turnip greens, collards, mustard greens, broccoli) the range is from about 75 to 200.

An especially deleterious feature of the composition of New Zealand spinach is its high oxalate content. Oxalic acid in chemical combination with calcium forms a very insoluble compound. This is likely to be true also of magnesium (Pierce and Appleman, '43). Since New Zealand spinach has been reported to contain an unusually high percentage of oxalic acid, sufficient, perhaps, to more than precipitate and form insoluble, indigestible oxalates with the entire supply of the plant's calcium and magnesium, the degree to which the oxalate would balance stoichiometrically and make insoluble these two basic minerals in the plant may be determined by converting to milliequivalents the percentages of oxalate, calcium and magnesium given in table 1.

On such a chemically equivalent basis, the oxalate would exceed by several times the calcium at all fertility levels. Also, the total oxalate would be far in excess of the plant's calcium and magnesium combined. Thus is left a sizable surplus of soluble oxalates for possible combination with calcium derived from other foods in the diet. If enough calcium from other sources such as milk were to be added to balance the excess oxalate, it would require from about four to six times as much as the crop itself contributes. With the problems of calcium availability in New Zealand spinach, there are involved not only initially larger quantities of oxalate, but also amounts of calcium and magnesium native in the crop, which are considerably lower than those in any related vegetables (Schroeder and Albrecht, '42; Wittwer, Albrecht and Goff, '45).

SUMMARY AND CONCLUSIONS

New Zealand spinach, compared with other leafy vegetables as a "protective" food, is a poor source for vitamin C.

[1] Vitamin C given in milligrams per 100 gm. fresh weight, oxalate and mineral analyses in percentage of dry weight.

Its low calcium and magnesium concentrations with an extremely high oxalate content suggest a negative mineral contribution; there is likely a precipitation of quantities of calcium derived from other foods consumed, tantamount to from four to six times that furnished by the plant itself.

The response of the crop to nitrogen fertilization was (1) practically independent of the soil calcium, and (2) reflected by an increased yield of vegetation which generally became progressively inferior in nutritional value with each increment of nitrogen.

New Zealand spinach is at the bottom of the list of green leafy vegetables insofar as its vitamin C and calcium contributions are concerned.

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THE RELATION OF VITAMIN A INTAKE TO LENGTH OF LIFE, GROWTH, TOOTH STRUCTURE AND EYE CONDITION

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THREE FIGURES

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INTRODUCTION

The minimum vitamin A requirement has been defined by Smith ('42) as the amount of vitamin A calculated to a daily dose that just prevents clinical symptoms of vitamin A deficiency. This amount has been found adequate for good growth and general well-being but permits little or no storage. Goss and Guilbert ('39) on the basis of data obtained from vaginal smear records estimate the minimum vitamin A requirement for the albino rat at 1.8–2.2 units of vitamin A per 100 gm. body weight. This amount of vitamin A was said to be sufficient for normal growth.

Lewis et al. ('42) have shown that intakes up to 25 U.S.P. units of vitamin A per rat daily result in improved growth. There was no increase in growth when amounts greater than 25 units daily were fed. Baumann, Riising and Steenbock ('34) found that as little as one International Unit (I.U.) of vitamin A per rat daily was sufficient to cure xerophthalmia and restore growth but that 7 to 17 I.U. were required to produce storage in the liver. Horton et al. ('41) conclude from their work that about 20 I.U. daily per rat were sufficient to main-

tain the blood level of vitamin A but were not sufficient for storage.

Irving and Richards ('39) have postulated that the vitamin A requirement of the rat increases with age, 3 I.U. daily being sufficient to maintain normal colored teeth in the rat up to 52 days of age. However, at 180 days of age, the teeth were not entirely normal in the animals receiving 3 I.U. daily.

Guilbert and Hart ('35) believe that vitamin A requirements are a function of the body weight. The majority of experiments reported in the scientific literature were conducted without consideration of the increasing body weight of the rat. Therefore, the rat fed on a straight daily dose basis would receive less vitamin A per unit of body weight as his size increased.

McCay, Crowell and Maynard ('35) have shown that the life span of retarded growth animals exceeds that of animals which have grown to maturity rapidly. These results were obtained with animals whose growth was retarded by a deficiency of calories. That slower growth due to suboptimal vitamin A levels is not compatible with maximum life expectancy is demonstrated by the experiments of Sherman and MacLeod ('25). These experiments, however, do not provide data on the quantitative vitamin A requirement of the albino rat for maximum life expectancy.

In view of the varying data it seemed advisable to carry on further investigations on the vitamin A requirement of the rat, administering the vitamin A on a body weight basis and carrying the animals throughout the entire life span on the experimental diets. Experiments were designed with the following questions in mind:

1. How does the minimum vitamin A requirement as previously determined compare with the vitamin A requirement for maximum length of life?

2. Is the minimum level of vitamin A previously established from vaginal smear records sufficient for normal growth, normal tooth structure and for normal eyes?

EXPERIMENTAL PROCEDURE

Young rats 28 days of age were placed on the U.S.P. XII vitamin A-free diet until signs of vitamin A deficiency such as loss in weight and xerophthalmia occurred. They were then assembled into four groups as evenly as possible with regard to age, weight, sex, tooth condition, and general condition. The four groups then received in addition to the vitamin A-free diet the following vitamin A supplements per 100 gm. body weight daily: group A — 1 U.S.P. unit, group B — 2 U.S.P. units, group C — 4 U.S.P. units, group D — 20 U.S.P. units. In terms of the requirement postulated by Goss and Guilbert ('39) this would be, respectively, ½, 1, 2, and 10 times the minimum.

The vitamin A-free diet had the following percentage composition: casein [1] (vitamin-free), 18.0; starch, 65.0; yeast — dried,[2] 7.5; irradiated yeast,[3] 0.5; salt mixture (U.S.P. no. 1), 4.0; and cottonseed oil, 5.0.

The source of vitamin A was distilled A ester [4] of approximately 300,000 U.S.P. vitamin A units per gram. The same refrigerated stock of vitamin A was used throughout the experiment. The vitamin A potency was determined spectrographically and confirmed by bioassay. Dilutions were freshly made each week and administered orally by syringe. The original stock solution was rechecked from time to time during the experiment to insure its maintenance of potency.

The animals were weighed weekly and the size of the dose adjusted for the next week on the basis of this weight. Records of the appearance of the teeth and general condition of the animals were made at monthly intervals. All animals were maintained on this general regime for the entire life span.

After starting vitamin A supplements the teeth of the animals in groups A, B, C, and D were examined at monthly intervals. Records were kept by colored drawings of the upper

[1] Labco Casein — The Casein Company of America, Inc., New York City.
[2] Strain G — Anheuser-Busch, Inc., St. Louis, Missouri.
[3] Type 9–F — Standard Brands, Inc., New York City.
[4] Distillation Products, Inc., Rochester, New York.

incisor teeth of each animal. Observations were made with respect to (a) chalky appearance, (b) light yellow or white color (i.e., lack of normal orange pigmentation), (c) black discoloration and (d) any combination of these abnormalities. An arbitrary system of grading the abnormalities was adopted. Incisor teeth exhibiting a deep orange pigmentation and a translucent appearance were considered normal and graded 0. Chalky white teeth with black pigmentation were graded 4. Intermediate degrees of abnormality were graded 1, 2, or 3 depending on the estimated severity of the condition. These gradings may not be entirely indicative of degree since there was no way of knowing whether black discoloration or lack of pigment constituted the more severe change.

The eye condition of the animals in all groups was noted every 2 weeks with regard to presence or absence of water, red exudate, corneal ulcers, swelling, etc. In judging the eye conditions an opaque cornea due to a healed corneal ulcer was not considered abnormal since it represented a healing of the abnormal condition even though the eye could not be returned to normal.

It was believed that the cottonseed oil in the diet would provide sufficient vitamin E. However, as the experiment progressed increasing evidence on the effects of vitamin E made it seem desirable to determine this so group C was subdivided when the experiment had been in progress about 1 year. One-half the animals of group C were continued on the original supplement of vitamin A (4 U.S.P. units per 100 gm. body weight). The remainder of the group received, in addition to this dose of vitamin A, 0.1 mg. of mixed tocopherols (from mixed tocopherol [4] solution) daily per 100 gm. of body weight. This is well above the minimal dose of vitamin E established by Mason ('40) for the albino rat. Group C was chosen for the illustration of any possible synergistic effect of added vitamin E since this was proving to be a borderline group. The animals were surviving well, growth was fair, and the general appearance was fair. The teeth were showing

some abnormalities and in general the animals were inferior to group D.

RESULTS

The data on the effect of the addition of vitamin E to group C after 1 year on the experimental diet are presented in table 1. Apparently the additional vitamin E had no beneficial effect on the tooth structure, the animals in both groups showing about the same increasing severity of abnormalities. Although both the average age at death and the average maximum weights attained were slightly greater for the animals receiving vitamin E, inspection of the probable errors for

TABLE 1

Effect of added vitamin E on group C.

SUB-GROUP	NO. ANIMALS	VIT. A PER 100 GM. BODY WEIGHT DAILY	ADDED TOCOPHEROLS PER 100 GM. BODY WEIGHT	TOOTH CONDITION		AVE. MAX. WEIGHT ATTAINED	AVE. AGE AT DEATH
				Start of E therapy	5 mo. later		
		U.S.P. units	*mg.*			*gm.*	*days*
C_0	8	4	0	1.9	2.7	334 ± 19	589 ± 24
C_1	7	4	0.1	1.3	2.2	356 ± 19	629 ± 28

these two values indicates that these differences are not significant. The above data indicate that the basal diet was not improved by additional vitamin E. We have therefore dealt with group C as a single group in considering the rest of the data.

For convenience the results of increasing vitamin A intake for the four groups are discussed under the headings of Longevity, Growth, Teeth, and Eyes.

Longevity. The data of table 2 show that the average age at death of the animals increases with the amount of vitamin A fed. The average age at death of thirty-five similarly treated animals on the same diet receiving no vitamin A and serving as negative controls in routine assays was 69 ± 0.9 days. One U.S.P. unit of vitamin A although insufficient for maintenance of life over a long period does increase the life span significantly (i.e., from 69 ± 0.9 to 80 ± 2 days).

The animals in group D receiving the highest level of vitamin A show an average age at death (649 days) which is comparable to that obtained by other workers using synthetic diets. McCay, Crowell and Maynard ('35) report an average age at death for both sexes of 677 days in their fast-growing group.

In figure 1, the mortality data for the four groups are charted at 100-day intervals along with the body weight curves. Inspection of these data reveals that differences in life span between the 4- and 20-unit levels only became apparent after

TABLE 2

Average age at death of animals receiving varying amounts of vitamin A.

GROUP	VITAMIN A PER 100 GM. BODY WEIGHT DAILY	NO. ANIMALS STARTED		AVE. AGE AT DEATH		AVE. AGE AT DEATH ALL ANIMALS
		♂	♀	♂	♀	
	U.S.P. units			*days*		*days*
A	1	9	14	81	79	80 ± 2
B	2	9	10	204	260	234 ± 18
C	4	10	10	478	564	521 ± 28
D	20	10	10	622	675	649 ± 30

the animals are 200 to 250 days of age. In other words the higher vitamin A intake was actually effective in prolonging the latter half of the life span and not in reducing mortality during the early part of life. It might be postulated that there is an increased requirement for vitamin A at this time although more data would be required to substantiate this conclusion. Irving and Richards ('39) have suggested that the vitamin A requirement of the rat increases with age. However, in their experiments the increasing body weight of the rat was not compensated for by increasing vitamin A intake.

Growth. The growth curves for the four groups of rats during the experiment are shown in figure 1. It is obvious that 1 U.S.P. unit of vitamin A per 100 gm. body weight is insufficient for prolonged maintenance of life or growth in the albino rat. Moderate growth was obtained at the 2-unit level (group

B) and growth was progressively better at the 4- and 20-unit levels.

Teeth. The effect of vitamin A deficiency on the structure of the tooth of the albino rat has been described in detail by Wolbach and Howe ('33) and by Schour et al. ('41). Irving and Richards ('39) have found 3 I.U. of vitamin A daily insufficient to maintain entirely normal teeth in older animals.

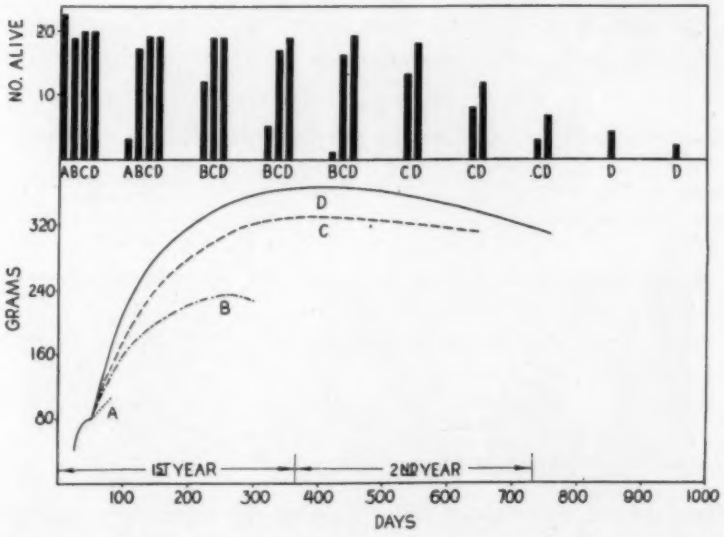

Fig. 1 Body weight curves of rats receiving different amounts of vitamin A per 100 gm. of body weight. The columns at the top show the number alive in each group at 100-day intervals. A — 1 U.S.P. unit, B — 2 U.S.P. units, C — 4 U.S.P. units, D — 20 U.S.P. units.

In our experiments the incisor teeth became uniformly chalky (opaque) during the preliminary depletion period and the orange pigmentation changed from slightly lighter to nearly white in some cases. Frequently a black mottling of the teeth was observed. We note that other workers have observed the chalkiness and the lack of pigmentation but we have seen no mention in the literature of a black discoloration

accompanying vitamin A deficiency. This black discoloration appeared frequently in groups A, B, and C of this experiment but was of rare occurrence in group D. We have made no detailed histological studies of the discoloration. The symptom also appears frequently in animals depleted for vitamin A assays in this laboratory. It does not resemble the mottling caused by excessive fluorine in the diet but from preliminary observations resembles a deposit of pigment.

In table 3 the average tooth condition of the four groups after 26 weeks of supplement has been summarized. This

TABLE 3

Condition of eyes and teeth of albino rats receiving different amounts of vitamin A.

GROUP	NO. ALIVE	TOOTH CONDITION AT 26 WEEKS	TOOTH GRADE AVE.	EYE CONDITION AT 26 WEEKS
A	..	All abnormal [1]	..	96% abnormal [1]
B	15	All showed severe abnormalities	3.33	74% showed abnormalities
C	19	80% showed abnormalities	2.05	40% showed abnormalities
D	19	95% normal — very slight abnormality in 1 case	0.08	95% normal — 1 animal abnormal

[1] Condition at death — prior to 26 weeks.

time period was chosen since most of the animals in groups B, C, and D were alive at this time and since it was typical of the entire period for each group. It is apparent from the data that the severity of the tooth abnormalities is inversely related to the vitamin A intake. Of the levels studied normal teeth were produced only at the 20-unit dosage. It is entirely possible that a level between 4 and 20 units would have sufficed. Photographs of the various degrees of tooth abnormalities observed in this experiment are to be found in figure 2.

Eyes. In table 3 the average eye condition at 26 weeks or at death (whichever occurred first) has been recorded. The same general picture is again borne out — a decrease in abnormali-

ties with an increase in vitamin A intake. Normal eyes in 95% of the cases were obtained only at the 20-unit level. The one case of eye abnormality in this group was not necessarily due to A deficiency for animals in this colony on a high A intake occasionally exhibit a reddish exudate from the eye which persists over long periods.

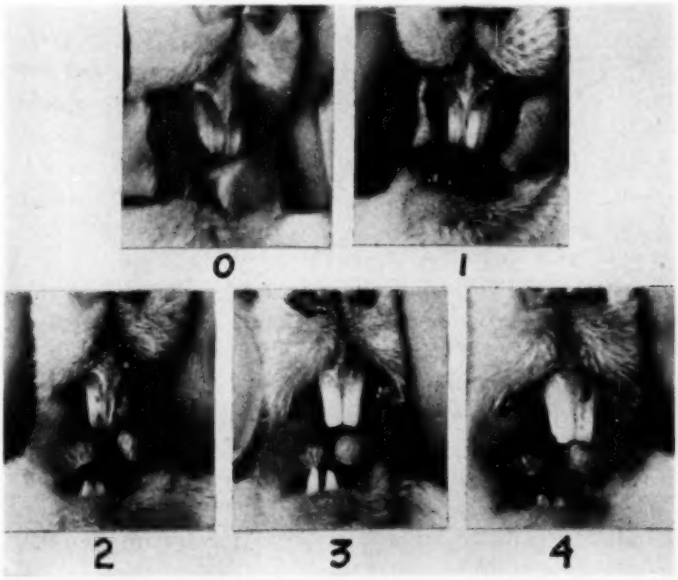

Fig. 2 Teeth showing various degrees of abnormality.

0 Normal, translucent, orange-pigmented tooth of rat 43♂ — group D. 20 U.S.P. units of vitamin A per 100 gm. of body weight.

1 Opaque, light-yellow tooth of rat 39♀ — group C — 4 U.S.P. units of vitamin A per 100 gm. of body weight.

2 Light-yellow color tooth with slight black mottling of rat 13♂ — group C — 4 U.S.P. units of vitamin A per 100 gm. of body weight.

3 Chalky-white tooth of rat 50♀ — group B — 2 U.S.P. units per 100 gm. of body weight.

4 Chalky-white tooth with black mottling of rat 11♂ — group B — 2 U.S.P. units per 100 gm. of body weight.

DISCUSSION

In these experiments an allowance of vitamin A for the albino rat of 2 U.S.P. units per 100 gm. of body weight allowed only suboptimal growth and cut the life expectancy to nearly one-third of that attainable on the same diet with increased vitamin A intake. The 2-unit level was also insufficient for normal tooth structure and healthy eyes.

From our data it may be estimated that the vitamin A requirement of the albino rat lies between 4 and 20 U.S.P. units daily per 100 gm. of body weight. Although no levels higher than 20 were studied, it is believed that this level equals or exceeds the requirement since (1) normal tooth and eye structure was produced at this level and (2) a five-fold increase in vitamin A above the 4-unit level produced approximately a 20% increase in body weight and a 25% increase in life span, whereas the 4-unit level compared with the 2-unit level (i.e., only a two-fold increase of vitamin A) produced an increase of 120% in life span and an increase of 20% in body weight. This has been graphically represented in figure 3 and from this graph the vitamin A requirement of the albino rat may be estimated at about 10 U.S.P. units per 100 gm. of body weight per day (i.e., the point approaching the flat portion of the curve). The requirement for normal teeth and eyes apparently lies in the same general range. The data indicate that optimal growth might be used as a criterion for the establishment of a vitamin A requirement adequate for all physiological functions.

This value of 10 U.S.P. units greatly exceeds the requirement of 1.8–2.2 U.S.P. units per 100 gm. body weight estimated from the data on vaginal smear records (Goss and Guilbert, '39). Better agreement is found with the values which just permit storage (7 to 17 U.S.P. units per rat found by Baumann, Riising and Steenbock, '34) or which permit optimum growth and very slight storage (25 U.S.P. units per rat as determined by Lewis et al., '42). Horton's ('41) work on the amount of vitamin A necessary to maintain blood levels also indicates that 20 I.U. of vitamin A represents the daily re-

quirement of the rat but does not allow storage. Horton reports 4 I.U. per rat daily as sufficient for the cure of xerophthalmia. In our experiment, 4 U.S.P. units per 100 gm. body weight gave relief of xerophthalmia in 60% of the animals.

It is believed that in establishing nutritional formulae a value for vitamin A should be used which is adequate to cover all physiological functions. Our experiments indicate that for

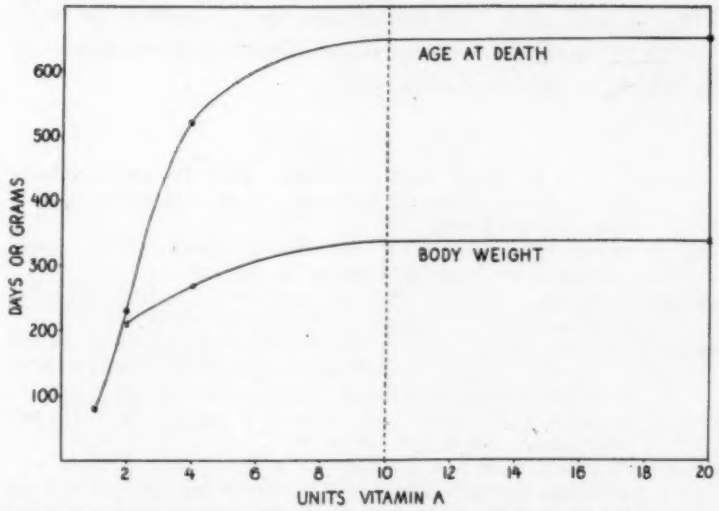

Fig. 3 Relationship between vitamin A intake and body weight. x————x body weight of rats at 26 weeks. Relationship between vitamin A intake and age at death. o————o average age at death of rats.

the albino rat this value is approximately 10 U.S.P. units of vitamin A per 100 gm. body weight. Calculation of the data of other workers to a body weight basis indicates fair agreement with this value.

SUMMARY

On a vitamin A-free diet the life span of albino rats increases with increasing vitamin A intake at levels of 1, 2, 4 and 20 U.S.P. vitamin A units per 100 gm. of body weight.

The effect of increasing vitamin A levels is reflected similarly on the four physiological criteria studied — life span, growth, tooth structure, and eye condition.

The vitamin A requirement of the albino rat estimated on the basis of maximum life span and optimal growth may be approximated at 10 U.S.P. units of vitamin A per 100 gm. of body weight. This is about five times the minimum requirement previously estimated on the basis of vaginal smear records.

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NICOTINIC ACID DEFICIENCY IN TURKEY POULTS AND THE OCCURRENCE OF PEROSIS[1]

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ONE FIGURE

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Briggs, Mills, Elvehjem, and Hart ('42) have shown that young chicks require a dietary source of nicotinic acid when fed highly purified diets. This finding has been extended to another avian species, the turkey, and the results, presented in this paper, show that the poult also requires nicotinic acid in the diet. Symptoms of a deficiency include poor growth, inflammation of the mouth, diarrhea, and perosis.

This observation extends the ever-growing list of animals which have been shown to require a dietary source of nicotinic acid since the discovery of the vitamin nature of this compound by Elvehjem, Madden, Strong, and Woolley ('38).

EXPERIMENTAL

Day-old turkey poults (crossbred), obtained from the Poultry Department, were divided into uniform groups and reared

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in electrically heated battery cages with raised wire floors. Feed and water were given ad libitum. Weighings and other observations were made weekly and the experiments conducted for a 4-week period.

The basal ration, low in nicotinic acid but complete in all other nutrients, was composed as follows: glucose ("Cerelose") 61.4, casein (Vitamin Test GBI) 18, gelatin 10, l-cystine 0.3, soybean oil 4, and Salts 1 M [2] 6. Each 100 gm. also contained the following amounts of vitamins: thiamine HCl 0.4 mg., riboflavin 0.8 mg., Ca pantothenate 2.0 mg., choline HCl 250 mg., pyridoxine HCl 0.6 mg., biotin 0.02 mg., i-inositol 100 mg., p-aminobenzoic acid 0.2 mg., 2-methyl-1, 4-napthoquinone 0.1 mg., and alpha-tocopherol 0.5 mg. Vitamin A and vitamin D_3 (1200 U.S.P. units and 170 A.O.A.C. units, respectively) were fed by dropper weekly. Unidentified factors were supplied by a norite eluate concentrate of liver, containing vitamins B_{10}, B_{11}, and B_c, prepared from Liver Fraction L (Wilson) by the method of Hutchings, Bohonos, and Peterson ('41). The amount of this fraction added was equivalent to 4% of original Liver Fraction L (or about 0.2% dry matter). The amount of nicotinic acid in the basal ration was not determined but since it is nearly identical with the ration used by Briggs et al. ('42) it probably contained not more than 0.3 mg. of nicotinic acid per 100 gm.

RESULTS

A summary of four different experiments is given, for the sake of simplicity, in table 1. It can be seen that poults receiving the basal ration grew poorly, with some deaths, and developed an inflammation of the mouth parts (similar to chick blacktongue). In addition, poults receiving the basal

[2] Salts 1 M are composed of the following ingredients by weight:

$CaCO_3$	150.0	$MgSO_4 \cdot 7H_2O$	50.0	$ZnCl_2$	0.2
K_2HPO_4	90.0	$Fe(C_6H_5O_7)_2 \cdot 6H_2O$	14.0	$CuSO_4 \cdot 5H_2O$	0.2
Na_2HPO_4	73.0	$MnSO_4 \cdot 4H_2O$	4.1	H_3BO_3	0.09
$Ca_3(PO_4)_2$	130.0	KI	0.4	$CoSO_4 \cdot 7H_2O$	0.01
NaCl	88.0				600.0

When fed at a level of 6%, the mixture supplies 1.11 gm. of calcium, 0.58 gm. of phosphorus, and 0.01 gm. of manganese per 100 gm. of ration.

ration had poor feathers, diarrhea, low feed consumption, and low efficiency of feed utilization (.334 [3] as compared with the control value of .626).

As the level of nicotinic acid was increased in the ration, growth likewise increased until a level of 3 to 5 mg. per 100 gm. was reached. There was a marked decrease in the incidence of inflammation of the mouth with the addition of 1 mg. of nicotinic acid per 100 gm. of ration and higher amounts prevented this trouble entirely. Thus, a greater level of nicotinic acid is required for growth than for the prevention

TABLE 1

The effect of nicotinic acid intake on growth, inflammation of the mouth, and perosis in turkey poults.

GROUP	SUPPLEMENT TO BASAL RATION (NICOTINIC ACID LOW)	NO. STARTED	NO. ALIVE AT 4 WKS.	AVERAGE WEIGHT AT 4 WEEKS (WITH RANGE) GM.	NO. WITH INFLAMMATION OF MOUTH	NO. WITH PEROSIS
1	None	19	13	114 (84–144)	14	6
2	1 mg. nicotinic acid/100 gm.	6	6	154 (120–190)	1	3
3	2 mg. nicotinic acid/100 gm.	13	11	267 (170–358)	0	9
4	3 mg. nicotinic acid/100 gm.	13	13	332 (175–433)	0	4
5	4 mg. nicotinic acid/100 gm.	7	7	345 (300–430)	0	0
6	5 mg. nicotinic acid/100 gm.	20	20	350 (242–466)	0	1
7	10 mg. nicotinic acid/100 gm.	6	6	370 (287–405)	0	0

of mouth inflammation. The 10-mg. level of nicotinic acid gave slightly better growth than the 5-mg. level. This difference, however, is not thought to be significant. A larger number of poults would have to be used in order to establish the exact level of nicotinic acid required.

Perosis. It will be noted in table 1 that the poults receiving low levels of nicotinic acid had perosis (fig. 1). The incidence of perosis was highest in the groups receiving 2 mg. of nicotinic acid and was also more severe in these groups, often involving an actual slipping of the tendon of Achilles from its

[3] $\frac{\text{Total weight gained}}{\text{Total feed consumed}}$

condyle. Higher levels of nicotinic acid caused a marked reduction of incidence of perosis. It is evident, therefore, that nicotinic acid is required by the poult for the prevention of perosis in addition to manganese (Ringrose, Martin and Insko, '39), choline (Jukes, '40), and biotin (Patrick, Boucher, Dutcher and Knandel, '43).

Fig. 1 A typical case of perosis in the turkey poult due to too low a level of nicotinic acid in the diet.

DISCUSSION

The amount of nicotinic acid required by the turkey poult (at least 3 to 5 mg. of nicotinic acid per 100 gm. of ration) is not less than two or three times higher than the minimum level required by the White Leghorn chick (Briggs et al., '42). This is not especially surprising since it has been found that the turkey poult's requirements for most of the other vita-

mins is higher than that for the chick. It should be emphasized that the nicotinic acid deficiency in the poult was produced by the use of a highly purified diet and that the results do not show whether or not the requirement for nicotinic acid by poults receiving practical rations is the same.

The perosis obtained in these experiments might be expected from studies with young chicks which have shown that a nicotinic acid deficiency in a ration, adequate in all other respects, may result in perosis (Briggs, Luckey, Tepley, Elvehjem and Hart, '43, and unpublished data). The occurrence of perosis due to this deficiency may explain the perosis obtained in poults by Patrick et al. ('43) which they attribute to an unrecognized factor in yeast "which can be adsorbed on fuller's earth or norite and subsequently eluted with ammonium hydroxide." The simplified rations which they used appear to be low in nicotinic acid. Evans, Rhian and Draper ('43) have reported that turkey poults, receiving a natural type of diet, require another factor or factors for the prevention of perosis other than manganese and choline.

It is recognized that still other factors may be required for the prevention of perosis. If still another unidentified factor (or factors) is required, it must be contained in the norite eluate concentrate of the basal ration (or as an impurity in the other purified ingredients).

SUMMARY

Turkey poults, when fed a highly purified diet containing all necessary nutrients except nicotinic acid, grew poorly and developed certain deficiency symptoms such as inflammation of the mouth, diarrhea, low feed consumption, poor efficiency of feed utilization, poor feathering, and perosis. The perosis in the deficient birds occurred in spite of ample manganese, choline, and biotin in the diet. All symptoms were prevented by the addition of 3 to 5 mg. of nicotinic acid per 100 gm. of ration. Higher levels of nicotinic acid, however, may be needed for optimum growth.

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FACTORS AFFECTING THE DIETARY NIACIN AND TRYPTOPHANE REQUIREMENT OF THE GROWING RAT [1]

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ONE FIGURE

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That the dietary requirement of a single nutrient can be rather variable and dependent upon the character and quality of the accompanying nutrients is becoming increasingly evident. Such interplay of nutrients is classically exemplified by the sparing action of fat on thiamine (Evans, Lepkovsky and Murphy, '34) and the increased dietary riboflavin requirement for the rat on high fat rations (Mannering, Lipton and Elvehjem, '41). The effect of the type of carbohydrate on intestinal synthesis of undifferentiated B complex factors is evident from the coprophagy studies of Guerrant, Dutcher and Tomey ('35) and Guerrant, Dutcher and Brown ('37). The effect of these extrinsic variables which so markedly alter the physiology of the organism, has resulted in an increased interest in the importance of these factors to the host. Krehl, Teply and Elvehjem ('45) have shown that when corn is added

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to synthetic rations, the nicotinic acid requirement of the dog is increased. In addition it has been demonstrated (Krehl, Teply and Elvehjem, '45) that corn is an etiological factor in the production of a nicotinic acid deficiency in the rat, a species which heretofore had not been shown to require a dietary source of this vitamin. This deficiency was further complicated by the fact that increased levels of casein eliminated the deleterious effect and that tryptophane alone was effective on low casein diets (Krehl, Teply, Sarma and Elvehjem, '45). From these results it is evident that an interrelationship between a vitamin (nicotinic acid) and an amino acid (tryptophane) exists. Although this phenomenon appears to be new in animal nutrition it is not so in bacterial nutrition as Stokes and Gunness ('45) have shown that pyridoxine and pyridoxamine and certain amino acids have a dual role for some Lactobacilli.

This investigation was carried out to obtain further information on the interchangeable role of nicotinic acid and tryptophane and the relationship of other ration components to the dietary tryptophane requirement.

EXPERIMENTAL AND RESULTS

General

The composition of the nicotinic acid low basal rations used is given in table 1. Vitamins were incorporated in all these rations, unless otherwise indicated, at the following levels: thiamine chloride 0.2, riboflavin 0.3, pyridoxine HCl 0.25, calcium pantothenate 2.0, choline chloride 100, inositol 10, 2-methyl-naphthaquinone, 0.1 and biotin 0.01 mg. per 100 gm., respectively. Halibut liver oil diluted 1:2 with corn oil was fed at a level of 2 drops per week, with α-tocopherol included at 0.5 mg. per drop. A norite eluate of solubilized liver extract, prepared so as to contain practically no nicotinic acid was fed where indicated. This preparation was made by adjusting the norite eluate to pH 3, followed by the addition (in the cold) of absolute ethanol to make a concentration of 90% by

TABLE 1

Composition of basal rations used.

RATION CONSTITUENT	RATION NO.																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Casein, gm.[1]	15	20	15	15	15	15	15									9	9	9
Gelatin, gm.										10	10	10	10	10	10			
Wheat gluten, gm.[2]										15	15	15	15	20				
Corn starch, gm.													68				84	
Dextrin, gm.			78								68					84		
Glucose, gm.				78								68						
Lactose, gm.					26													
Sucrose, gm.	78	73			52	55	55	42	52	68				68				84
Corn oil, gm.	3	3	3	3	3		25		6	3	3	3	3	3	2	3	3	3
Butter fat, gm.[3]						25												
Salts IV, gm.[4]	4	4	4	4	4	5	5	2	2	4	4	4	4	4	3	4	4	4
l (—) Cystine, gm.	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15							0.2	0.2	0.2
Klim, gm.[5]								56										
Skim milk powder									40									
Refined wheat flour[6]															85			

Generous levels of vitamins (see text) were supplied in all rations.

[1] Extracted 3 × with hot 95% ethanol.

[2] Fed at a level to allow 15% protein.

[3] Washed twice with hot water.

[4] Phillips and Hart ('35).

[5] A commercial whole milk powder.

[6] Unenriched refined wheat flour.

volume. The voluminous precipitate was filtered off, redissolved in the least amount of water necessary, the pH readjusted to 3, and the precipitation repeated as above. By this means nearly all of the nicotinic acid was removed with a minimum loss of "folic acid" activity. The corn products

TABLE 2

Protein, tryptophane and nicotinic acid contents of dietary supplements.

SUPPLEMENT	PROTEIN, TRYPTOPHANE AND NICOTINIC ACID CONTENT OF SUPPLEMENT		
	Protein (N× 6.25)	Tryptophane	Nicotinic acid
	%	*mg.%*	*mg.%*
Corn grits	9.0	25	0.65
Yellow corn	8.5	52	2.28
White corn	9.2	55	2.25
Ethanol extracted yellow corn	7.2	43	1.30
Polished rice	7.5	75	1.40
Soybean flour	50.0	600	2.00
Whole wheat	15.0	150	5.50
Refined wheat flour [1]	13.5	108	1.0
Whole rye flour	12.5	125	1.05
Rolled oats	12.5	150	1.05
Skim milk powder	37.5	450	1.05
Klim [2]	26.8	322	0.66
Whole milk	3.4	54	0.08
Casein	100.	1200	< 0.01
Wheat gluten	88.	800 [3]	1.62
Gelatin	105.	0.0	< 0.01
Corn starch	....	.....	...

[1] Unenriched white flour.

[2] A commercial whole milk powder.

[3] Calculated as 0.8%, based on 100% protein, calculated to 16% nitrogen.

and other cereals (table 2) were added to the extent of 40% of the entire basal ration. The vitamin and cystine levels were maintained as previously indicated in both the basal and supplemented rations. The counteracting effect of nicotinic acid and other substances was tested by incorporating them in the supplemented ration unless otherwise specified.

All the crude materials were ground to a consistency comparable to the rest of the ration and analyzed for crude protein ($N \times 6.25$) and nicotinic acid (table 2). The protein and nicotinic acid content of the various rations presented in table 3 are calculated on the basis of analytical results given in tables 2 and 3. The tryptophane values given in tables 2 and 3 are based on the best and latest available analytical data given by Block and Bolling ('43, '45). All rations were fed ad libitum to weanling rats of the Sprague Dawley strain. The growth period employed was 5 weeks unless otherwise indicated, and the growth results are presented in grams gained per week with the range in parentheses. The growth data obtained in the various experiments are presented in table 3.

Although the results are given only for the sucrose basal, growth was very good (28–30 gm. per week for 5 weeks) irrespective of the basal ration used (table 1) with the exception of rations 10 through 18 (to be discussed later). A growth performance markedly below that obtained on any of the basal rations then must be considered due to the deleterious action of the supplement used. To generalize further the addition of 1 to 1.5 mg.% of nicotinic acid (or 50 mg.% of tryptophane) to a corn supplemented ration invariably resulted in growth equal or superior to that obtained on the basal ration.

The results obtained with the basal rations, which contain practically no nicotinic acid clearly demonstrate that a dietary source of this vitamin is not required by the rat. Since the basal rations cannot be formulated to the same composition as the supplemented rations, results obtained with them are not to be interpreted as direct controls, but rather as valuable indices in interpreting growth results. Comparison of the growth performance on the corn-supplemented rations and these rations plus nicotinic acid or other corrective supplements, is however, a direct one.

TABLE 3

Growth results on various diets.

GROUP NO.	BASAL RATION (TABLE 1)	+ ADDED SUPPLEMENT (TABLE 2)	NICOTINIC ACID ADDED	TRYPTOPHANE ADDED	TOTAL PROTEIN	TOTAL TRYPTOPHANE	TOTAL NICOTINIC ACID	GAIN PER WEEK [4] (RANGE)
			+ or —	+ or —	%	mg.%	mg.%	gm.
1	1		—	—	15.0	180	< 0.01	28 (25–32)
2	1	Corn grits	—	—	12.6	118	0.27	7 (4–11)
3	1	Corn grits	+	—	12.6	118	1.27	29 (24–35)
4	1	Yellow corn	—	—	12.4	129	0.92	13 (11–16)
5	1	White corn	—	—	12.7	130	0.91	14 (7–16)
6	1	Polished rice	—	—	12.0	138	0.56	31 (27–34)
7	1	Soybean flour	—	—	29.0	348	0.80	26 (21–38)
8	1	Whole wheat	—	—	15.0	168	2.20	31 (31–31)
9	1	Refined wheat flour	—	—	14.4	151	0.40	29 (26–30)
10	1	Rye flour	—	—	14.0	158	0.41	26 (24–27)
11	1	Rolled oats	—	—	14.0	168	0.41	28 (26–29)
12	4	Corn grits	—	—	12.6	118	0.27	23 (18–29)
13	3	Corn grits	—	—	12.6	118	0.27	21 (13–26)
14	5	Corn grits	—	—	12.6	118	0.27	15 (12–17)
15	1	Corn starch	—	—	9.0	108	< 0.01	19 (16–22)
16	1	Corn grits + norite eluate [1]	—	—	12.6	118	0.27	6 (5– 8)
17	1	(low vitamins) [2]	—	—	15.0	180	< 0.01	21 (17–27)
18	1	Corn grits (low vitamins) [2]	—	—	12.6	118	0.27	7 (5–10)
19	1	Corn grits (low vitamins) [2]	+	—	12.6	118	1.27	20 (18–21)
20	4	(low vitamins) [2]	—	—	15.0	129	< 0.01	28 (26–31)
21	4	Corn grits (low vitamins) [2]	—	—	12.6	118	0.27	15 (11–18)
22	4	Corn grits (low vitamins) [2]	+	—	12.6	118	1.27	24 (21–29)
23	1	Corn grits (high vitamins) [3]	—	—	12.6	118	< 0.01	6 (6– 7)
24	1	Corn grits (high vitamins) [3]	+	—	12.6	118	1.77	20 (19–22)
25	1	Corn grits + 5 cc. milk/day	—	—	12.7	121	0.28	18 (9–26)
26	1	Corn grits + 10 cc. milk/day	—	—	12.9	124	0.28	23 (21–24)
27	9	Yellow corn	—	—	12.4	129	1.16	31 (26–34)
28	8	Corn grits	—	—	12.6	118	0.48	28 (25–31)
29	6	Corn grits	—	—	12.6	118	0.27	10 (9–12)
30	7	Corn grits	—	—	12.6	118	0.27	12 (10–14)
31	2	Yellow corn	—	—	15.4	165	0.92	30 (22–35)
32	1	Corn grits	—	+	12.6	168	0.27	31 (27–37)
33	1	Corn grits	—	+	12.6	218	0.27	33 (26–35)
34	1	Corn grits	+	—	12.6	118	1.77	33 (31–36)
35	10		—	—	25.0	120	0.25	7 (4–12) [5]
36	10		+	—	25.0	120	1.25	23 (21–28)
37	10		—	+	25.0	145	0.25	22 (17–26)
38	14		—	—	30.0	160	0.32	26 (24–29)
39	11		—	—	25.0	120	0.25	21 (17–24)
40	12		—	—	25.0	120	0.25	18 (18–18)
41	13		—	—	25.0	120	0.25	13 (8–18)
42	13		+	—	25.0	120	1.25	23 (19–26)
43	15		—	—	21.5	92	0.85	23 (18–26)
44	15		+	—	21.5	92	1.85	30 (29–31)
45	16		—	—	9	108	< 0.01	24 (21–28)
46	17		—	—	9	108	< 0.01	26 (24–28)
47	18		—	—	9	108	< 0.01	14 (12–15)
48	18		+	—	9	108	1.5	17 (17–18)
49	18		—	+	9	158	< 0.01	21 (19–23)

[1] Norite eluate $\cong$ 23 μg. B_c (*S. lactis* assay) per 100 gm. of ration.

[2] (low vitamins) — general vitamin level reduced to 60% of that given in text.

[3] (high vitamins) — general vitamin level increased to 3 times that given in text.

[4] Three animals used per group. Most experiments were repeated.

[5] 4-weeks growth period from weaning. This applies to all groups 35–49, inclusive — the rest of the items given in this column.

Effect of varieties of corn and other cereals on the growth of rats

Since corn grits are consumed in large quantities in the southern part of the United States and since they produce the most serious growth retardation (table 3) most of the work reported was done with this product. It can be seen (table 3, groups 4 and 5) that yellow or white corn do not retard growth as severely as corn grits. This may be due to the combined effect of the larger nicotinic acid and tryptophane content of these materials. Nicotinic acid in every case counteracted these growth depressions.

Corn grits which were heated (dry) in the autoclave for 1 hour at 15-pounds pressure still produced very poor growth. Ground white corn was reduced to the consistency of ordinary white flour by grinding for 24 hours in a ball mill, yet no significant improvement in growth could be observed as a result of this treatment.

In addition to corn, other cereals, namely polished rice, whole wheat flour, refined wheat flour, whole rye flour, whole rolled oats, and soybean flour were tested in a comparable manner. Good growth resulted in every case (table 3, groups 6 through 11). It is interesting to note that though polished rice, rolled oats, and rye, all contain significantly less nicotinic acid than yellow corn, yet they did not produce growth depression. This fact may in part be attributed to a higher tryptophane content.

The role of carbohydrate and vitamin level in counteracting the growth depressing action of corn

Inasmuch as it has been shown (Mannering, Orsini and Elvehjem, '44; Schweigert, McIntire, Henderson and Elvehjem, '45) that the type of carbohydrate used in a synthetic ration has a marked influence on vitamin synthesis in the intestinal tract, the effect of corn was tested when fed with sucrose, glucose, dextrin and lactose. When lactose was used the carbohydrate portion of the ration contained two-thirds

sucrose and one-third lactose, since rats fail to grow on a high lactose low-fat ration (Schantz, Elvehjem and Hart, '38). Starch was also used, but in this case it was added to the basal ration in the usual manner to the extent of 40%. The growth results obtained using various carbohydrates are shown in table 3, groups 12, 13 and 14, and it is evident that glucose, dextrin and to a lesser degree, lactose exert a marked influence in modifying the undesirable action of corn grits. These observations strongly indicate that corn produces alterations in the intestinal flora and that the type of carbohydrate used may be important in determining the extent of these changes. This theory is given additional support by the fact that when corn starch alone is added to the basal ration to the extent of 40% reasonably good growth is obtained (table 3, group 15), even though the nicotinic acid content is negligible and the 9% casein in this ration supplies several of the essential amino acids below the minimum indicated by Rose ('37). Furthermore, animals which received glucose, dextrin or lactose show a pronounced variability in growth not only between animals in the same group but at different points in the growth period, with poor growth occurring at first, followed by rapidly increasing growth from week to week. This variation might be due to a gradual favorable modification in the intestinal flora under the influence of the proper type of carbohydrate. Since Boutwell, Geyer, Elvehjem and Hart ('45) have shown that the level of vitamins is important in determining the growth of rats on high-fat rations, the vitamin level of the basal and corn-supplemented ration was lowered to 60% of the usual level. The results of this modification are noted in table 3, groups 17 through 22. When the vitamin level was lowered, glucose, which had previously exerted a protective action against corn, now failed to allow adequate growth on the corn-supplemented ration, although growth on the basal was normal. On the lowered vitamin level, growth on the sucrose basal was reduced from 28 to 21 gm. per week. Nicotinic acid was correspondingly

less efficient in counteracting the action of corn particularly when sucrose was used as the carbohydrate.

Since it is apparent that a lowered level of vitamins produces a significant effect, the vitamin level was tripled. It can be seen (table 3, groups 23 and 24) that no benefits were obtained from raising the general level of vitamins; in fact, the usual effectiveness of nicotinic acid was somewhat impaired. Norite eluate $\cong$ to 23 μg. of vitamin B_6 per 100 gm. of ration did not counteract the growth depression caused by corn; neither did p-aminobenzoic acid at a level of 25 mg. per 100 gm. of ration. Hence nicotinic acid alone of all the known members of the B complex factors seems to have a specific counteraction against the growth retarding effect of corn.

Milk and milk products as corrective agents against the effect of corn rations

Since Goldberger, Waring and Willets ('15) demonstrated that pellagra was a deficiency disease and advocated a good diet including adequate amounts of milk as a preventive (Goldberger and Tanner, '24), the value of milk in counteracting the deficiency caused by corn was ascertained.

When milk was fed at a level of 5 and 10 ml. per day as a supplement to the ration which contained corn, a marked beneficial effect was observed (table 3, groups 25 and 26). The addition of 5 and 10 gm. levels of whole milk powder per 100 gm. of ration also gave favorable results. In all cases where these small amounts of milk were used, growth was variable and quite often delayed, a phenomenon which also was seen on those carbohydrate rations which proved beneficial. In addition, the effect of these relatively small additions of milk could in every case be further improved by the addition of nicotinic acid. It should be emphasized, however, that the positive results that were obtained could not be related to the nicotinic acid or tryptophane content of the milk, but probably to an altered intestinal flora resulting from the feeding of milk. This difference in flora is obvious from a gross

observation of the enlarged cecum and of its contents. A skim milk powder ration (table 1, ration 9) and a whole milk powder ration (table 1, ration 8) were supplemented with yellow corn and corn grits, respectively. Excellent growth was obtained in both cases (table 3, groups 27 and 28). Again the results cannot entirely be attributed to the nicotinic acid or tryptophane content of these rations. These favorable results are probably due to a combination of several factors, including the high level of lactose.

Since Boutwell et al. ('45) have shown a nutritional difference between butter fat and corn oil, an experiment was designed to test the difference between these two fats in corn-supplemented rations. The high-fat basal rations (table 1, rations 6 and 7) which contain butter fat and corn oil, respectively, were supplemented with corn grits. Good growth was obtained with either basal ration, but neither butter fat or corn oil proved effective in counteracting the action of corn (table 3, groups 29 and 30).

The efficacy of tryptophane in counteracting the action of corn

That tryptophane is intimately concerned in the deficiency syndrome caused by corn has been demonstrated (Krehl et al., '45). Increased tryptophane ingestion, whether by increased protein level, or added l (—) or dl-tryptophane in amounts as low as 50 mg. per 100 gm. of ration is as effective as 1 or 1.5 mg. of nicotinic acid, in counteracting the deleterious effect of corn (table 3, groups 32, 33 and 34).

If nicotinic acid produces the observed effect by establishing a type of intestinal flora which favors the synthesis of protein (tryptophane) then small injections of nicotinic acid should have a limited action providing excretion into the intestine does not occur. If in the same manner, a tryptophane deficiency is the critical factor in this syndrome, the injection of this substance in amounts comparable to those effective in the

ration, should prove completely adequate in correcting the deficiency.

To test this idea four groups of rats (3 rats per group) that had received the corn supplemented ration were treated as follows: Group 1 received 0.1 mg. of nicotinic acid daily (this amount received daily in the diet is adequate), group 2 received 0.2 mg. of nicotinic acid daily while group 3 was given 5 mg. of tryptophane daily. The tryptophane and nicotinic acid supplements were injected each day subcutaneously. The fourth group was maintained untreated as a control. After 1 week the growth results obtained in grams gained per day were as follows: Group 1 receiving 0.1 mg. nicotinic acid, 2.4; group 2 getting 0.2 mg. nicotinic acid, 4.3; group 3 with 5 mg. tryptophane, 6.6; and group 4, the control, 0.55.

It is evident that the performance of animals receiving tryptophane injections is superior particularly when compared to the lower level of nicotinic acid. This line of evidence, while favoring the idea that a tryptophane deficiency is paramount in this problem, is not conclusive. Again we cannot be sure to what extent excretion of the injected nicotinic acid or tryptophane into the intestinal tract modifies the above results.

Counteraction of corn by various levels of nicotinic acid and nicotinic acid derivatives

Numerous levels of nicotinic acid were tested to determine the range of effectiveness of this vitamin in counteracting the deficiency caused by feeding corn. Levels ranging from 0.05 to 1.5 mg.% were tested and the results plotted in figure 1. It is apparent that the first level of nicotinic acid to show a significant effect is 0.4 mg.%. This level is based on sucrose as the carbohydrate and might be different when other carbohydrates are employed.

The following derivatives of nicotinic acid were also tested: nicotinamide, ethyl nicotinate, N-phenyl nicotinamide, trigonelline and nicotinamidemethochloride. These derivatives

were fed on a molar equivalent basis equal to 1.5 mg.% of nicotinic acid. Of these compounds, only nicotinamide and ethyl nicotinate were active. These results correlate with the action of these substances in curing blacktongue in the dog (Woolley, Strong, Madden and Elvehjem '38). N-phenyl nicotinamide appears to be inactive for the rat whereas it is

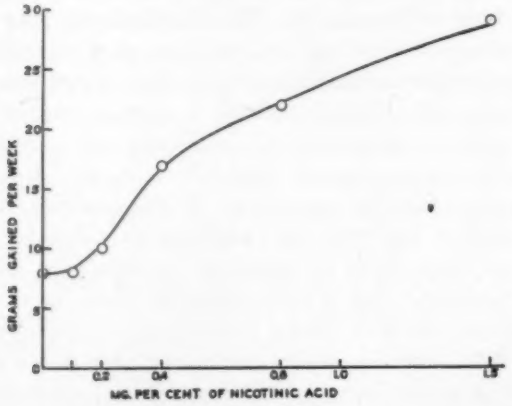

Fig. 1 The effect of various levels of nicotinic acid in counteracting the growth depression caused by corn.

about 25% as active as nicotinic acid for the dog. It is interesting to note that nicotinamidemethochloride is as inactive for the rat as for the dog (Teply, Krehl and Elvehjem, '45). This is in contradiction to the observation of Najjar, Hammond, English, Worden and Deal ('44), concerning the compound.

Additional observations on the deleterious effect of corn

Since all of the above experiments were done with male rats, the same experimental conditions were applied to a series of female rats in which case growth depression from corn and counteraction by nicotinic acid took place in a similar manner.

In addition to the growth depression observed in the corn-fed young growing rat, the animals appeared unkempt, with a rough fur coat and with occasional alopecia. Also the reddish accumulation of porphyrin like material on the paws, nose and whiskers of deficient rats described by Chick, Macrae and Worden ('40) was observed in all but a small percentage of the above animals.

To test the effect of corn supplemented rations on matured rats a group of adult males which weighed 250–285 gm. were given this ration for a period of 10 weeks. At the end of this time the animals weighed 330–407 gm. and showed no symptoms of any kind comparable to those seen in the young growing rat.

In an attempt to correlate an altered intestinal flora with differences in the amounts of nicotinic acid, "folic acid" and tryptophane found in the cecal contents of rats on the various diets, animals were sacrificed by decapitation, the cecal contents removed and autoclaved for 1 hour at 15-pounds pressure in 0.1 N KOH. The livers were removed at the same time, treated in a similar manner and analyzed for nicotinic acid. "Folic acid" was determined by the method of Teply and Elvehjem ('45), nicotinic acid by the method of Krehl, Strong and Elvehjem ('43) and tryptophane microbiologically by the omission of tryptophane and the inclusion of nicotinic acid in the medium of Krehl et al. ('43). The results of these analyses (table 4) clearly demonstrate that appreciable amounts of nicotinic acid are present in the cecal contents and that these amounts increase in those cases in which the carbohydrate used proved beneficial. The amounts of tryptophane and "folic acid" present in the cecal contents appear not to be markedly affected by the diet. It is rather difficult to deduce from this technique how much of the respective nutrient has been or is being made available to the host. The low nicotinic acid content of the livers of rats which received corn grits correlates well with retarded growth, and in every case in which nicotinic acid was added or good growth resulted from

TABLE 4

Analysis of cecal contents and liver.

BASAL RATION (TABLE 1)	+ SUPPLEMENT (TABLE 2)	NICOTINIC ACID ADDED	GRAMS GAINED PER WEEK [1]	ANALYSES OF CECAL CONTENTS [1]						LIVER ANALYSIS	
				Nicotinic acid		Tryptophane		"Folic acid"		Nicotinic acid	
				µg./gm.	Total	µg./gm.	Total	µg./gm.	Total	µg./gm.	Total
		+ or −		µg.	µg.	µg.	µg.	µg.	µg.	µg.	µg.
1		−	26	24	38	278	450	1.01	1.60	161	1626
1	Corn grits	−	6	30	44	269	387	2.27	3.09	106	420
1	Corn grits	+	26	39	67	245	430	2.17	3.60	159	1523
1	Corn grits [2]	−	7	30	38	317	397	1.45	2.23	109	398
1	Corn grits [2]	+	24	38	83	259	563	2.11	4.50	160	1345
1	Corn grits [3]	−	14	33	56	270	473	1.94	3.90	141	791
1	Corn grits [4]	−	19	34	66	265	515	1.95	3.45	135	1094
4	Corn grits	−	20	35	42						
4	Polished rice	−	32	46	79						
4	Rolled oats	−	36	53	126						
8		−	31	37	207						
8	Corn grits	−	28	36	134						
3	Corn grits	−	22	37	120						
5	Corn grits	−	15	37	163						

[1] Average of group of 3 rats.

[2] Norite eluate ≅ 11.5 µg. B_c per 100 gm. of ration.

[3] Plus 2 ml. fresh milk per day.

[4] Plus 5 ml. fresh milk per day.

feeding milk, the nicotinic acid content of the liver was elevated and was in the normal range. Whether the low values obtained are low enough to be a causative factor in the deficiency symptoms remains to be proven.

The influence of the type of carbohydrate and nicotinic acid on the dietary tryptophane requirement

Since it is evident that corn owes its deleterious action at least in part to the fact that it is deficient in tryptophane an effort was made to discover whether this action could be demonstrated with non-corn containing rations that were low in tryptophane. With this in mind rations 10 through 14 (table 1) were devised. Wheat gluten was the principle protein employed since it is low in tryptophane and gelatin was added to help compensate for other amino acid deficiencies, especially lysine. It is apparent from table 3, group 35 that rats fail to grow on this ration with sucrose as the carbohydrate unless nicotinic acid (group 36) or tryptophane (group 37) is added. Thus we find that corn is not unique in producing severe growth failure, and that the corrective interplay between nicotinic acid and tryptophane is not restricted to corn supplemented rations. Furthermore, we find a marked correlation in the protective action of dextrin and glucose in these rations, as in the corn rations, against the deleterious effect of a combined deficiency of tryptophane and nicotinic acid (table 3, groups 39 and 40). When corn starch was used as the carbohydrate in the wheat-gluten-gelatin ration (table 3, group 41) good growth was not obtained until nicotinic acid was added (group 42). It appears therefore that the efficacy of carbohydrate in counteracting the tryptophane deficiency decreases in the following order: dextrin, glucose, corn starch and sucrose. The addition of either nicotinic acid or tryptophane to rations which contain dextrin or glucose did not improve growth significantly which is indicative of their role in promoting an intestinal flora which adequately supplements a sub-optimum amount of dietary tryptophane. In this same

way, ration 15, (table 1) which contains 85% refined wheat flour resulted in good growth (table 3, group 43) despite its deficiency in tryptophane. The fair amount of nicotinic acid in this case is probably helpful which seems to be substantiated by the fact that additional nicotinic acid resulted in improved growth (group 44).

To further test the capacity of carbohydrate to modify the dietary tryptophane requirement, rations 16, 17 and 18 were prepared. Although the deficiency of tryptophane is paramount in these rations, other amino acids, particularly lysine, are also deficient. Despite this fact relatively good growth was obtained when dextrin or corn starch was employed as the carbohydrate (table 3, groups 45 and 46). Moreover, the addition of nicotinic acid or tryptophane did not benefit growth significantly. However, when sucrose was used as the carbohydrate, growth was poor and in this case the addition of nicotinic acid gave only partial improvement while tryptophane supported still better growth (table 3, groups 47, 48 and 49). This may be due to the fact that under these conditions sucrose is digested and absorbed so rapidly (Rabinowitch, '45) that it does not get into that part of the intestinal tract in which synthesis is most active.

Inasmuch as Albanese, Holt, Kajdi and Frankstone ('43) have shown that tryptophane plays an important role in maintaining an adequate level of total plasma protein and hemoglobin, analyses were made for these constituents. In addition, free plasma protein was determined. These results are compiled in table 5 and it is apparent that any direct correlation between growth failure and reduced plasma protein is made difficult due to the small differences obtained. Small differences are also evident in the hemoglobin values, while a consistent difference in the free plasma tryptophane is apparent between the animals which received corn and corn plus nicotinic acid, although both levels are in the normal range. At present it is impossible to explain the rather low plasma tryptophane values obtained on the wheat gluten

TABLE 5

Plasma protein, tryptophane and hemoglobin analysis.

BASAL RATION (TABLE 1)	+ SUPPLEMENT (TABLE 2)	NICOTINIC ACID ADDED	TRYPTOPHANE ADDED	AVERAGE GRAMS GAINED PER WEEK [1]	AGE FROM WEANING	PLASMA PROTEIN	HEMOGLOBIN	TRYPTOPHANE µG./ML. PLASMA
		+ or −	*+ or −*		*weeks*	*%*	*gm.%*	
1		−	−	27	4	5.5	9.9	
1	Corn grits	−	−	9	4	3.2	7.6	
1	Corn grits	+	−	27	4	5.3	10.5	
1		−	−	28	6	5.5	11.0	
1	Corn grits	−	−	8	6	4.7	13.0	
1	Corn grits	+	−	30	6	4.8	12.2	
1	Corn grits	−	+	31	6	5.3	12.6	
1		−	−	30	7	5.3	12.8	
1	Corn grits	−	−	9	7	4.4	10.7	
1	Corn grits	+	−	32	7	5.5	12.9	
1	Corn grits	−	−	8	7			14.7; 12.0
1	Corn grits	+	−	30	7			22.0; 18.2
10		−	+ .025%	22	4			18.0
10		+	+ .025%	23	4			17.0
10		−	−	7	4			13.3
10		+	−	23	4			16.7
11		−	−	21	4			8
11		+	−	24	4			5.5
13		−	−	13	4			7.0
13		+	+	23	4			7.0

[1] Average of group of 3 rats.

rations 11 and 13 which contained dextrin and corn starch, respectively.

DISCUSSION

While no complete explanation of the syndrome caused by feeding corn can be offered until the mechanism of the apparent interplay between nicotinic acid and tryptophane is elucidated, one can turn to the theory of an altered intestinal flora to explain most of the experimental observations. The effect of different carbohydrates on the growth depression might be explained on the grounds that they contribute to the establishment of an intestinal flora which is capable of synthesizing adequate amounts of the deficient factor. Of course, this does not explain why on nicotinic acid low synthetic rations, there is beneficial synthesis and normal growth, while changes in the intestinal flora and poor growth result from feeding corn. Again one might postulate a substance or substances in corn which combines with nicotinic acid or in some way makes it unavailable. Then too, Koser and Baird ('44) have demonstrated that a number of bacteria destroy nicotinic acid, and corn may well promote the development of such microorganisms. The fact that glucose which normally has a protective action against corn, gives much poorer protection when the vitamin level is lowered, again indicates an alteration of intestinal flora. This finding correlates with similar observations by Boutwell et al. ('45). Since small amounts of milk prove beneficial in counteracting the action of corn, one can reasonably assume a corrective action through re-establishment of a more favorable intestinal flora. The fact that the cecal contents are bulky, loose and quite liquid when milk is fed is probably due to the lactose.

Among the cereals tested corn seems to be quite specific in retarding growth of rats although rice, oats and rye which allow excellent growth are relatively poorer in nicotinic acid. The factor of a more adequate tryptophane supply probably accounts for this. It is also of interest to note that rats on the

rice-supplemented ration which contains only about 12% protein, grew 5 gm. per week more than did the animals on the soybean supplemented ration which contained 29% protein.

Although corn, like other cereals, contains starch, and although starch is a carbohydrate which seems to promote an intestinal flora favorable to the host, the supplementation of corn results in poor growth. The factors of low tryptophane and nicotinic acid concomitant with an unfavorable protein-carbohydrate relationship both as regards quality and quantity may be the cause of poor growth.

Despite the fact that both tryptophane and nicotinic acid are completely and separately active, the mechanism of this interplay is not understood. Upon analysis of the results in table 3 it is apparent than when corn is employed in the ration the dietary tryptophane is reduced to a level which does not provide growth. This situation can be corrected, however, by any of several means, namely by increasing the level of tryptophane to approximately 150 mg.% or the nicotinic acid to about 1.25 mg.%, or by using a type of carbohydrate (dextrin or glucose for example) which is known to promote intestinal synthesis. That these factors are clearly related and not a phenomenon peculiar to corn supplemented rations alone is evident from the fact that the same relationships hold true for the non-corn containing sucrose-wheat gluten-gelatin ration. A concomitant lysine or other amino acid deficiency in this ration may be responsible for poor growth, although experiments are in progress which indicate that the action of gelatin in these rations may be influential in creating an increased dietary requirement for tryptophane. Since relatively good growth is obtained on a ration which contains only 9% casein when dextrin or cornstarch is employed as the carbohydrate one must consider an alteration of the dietary requirement of amino acids, other than tryptophane, which are low in this ration. It appears further that the amino acid requirements as elaborated by Rose ('37) are subject to modification dependant on factors such as those described above.

For example the dietary tryptophane requirement of the growing rat seems to approximate 150 mg.% whereas Rose sets 200 mg.% as the minimum value. In this particular we are aware however, that although the tryptophane values used in the calculations are taken from the best accepted literature values, some of them may be in error.

Although the tryptophane deficiency encountered in the present study is sufficient to impair growth, the severe symptoms reported by Albanese ('45) such as corneal vascularization and cataract were not seen.

The observations made here indicate that a diet cannot be correctly and completely evaluated on the basis of its known chemical entities alone. Quantitative as well as qualitative relationships can be obscured by alteration of experimental conditions and interplay of nutrients. It is further evident that there is an increasing need for more information concerning the relationships between animal and bacterial nutrition.

SUMMARY

The addition of corn or corn grits to a nicotinic acid-low synthetic ration results in a profound growth retardation which can be completely counteracted by the inclusion of from 1–1.5 mg. of nicotinic acid or 50 mg. of tryptophane per 100 gm. of ration. Other cereals containing less nicotinic acid produce no such effect.

The kind of carbohydrate and the level of tryptophane influence the extent of the undesirable effect of corn. Glucose, dextrin and lactose were beneficial in their action. Small amounts of milk were also helpful in counteracting the growth depression but further benefits resulted from the addition of nicotinic acid. A low level of vitamins accentuated the deleterious action of corn.

Nicotinamide and ethyl nicotinate were as active as nicotinic acid on an equal molar basis while nicotinamidemethochloride, trigonelline, and N-phenylnicotinamide were inactive.

Non-corn rations low in tryptophane and nicotinic acid also gave poor growth when sucrose was used as the carbohydrate. The addition of tryptophane or nicotinic acid or the use of a carbohydrate which produced a favorable intestinal flora gave growth results comparable to those obtained with corn supplemented rations.

The factors affecting the dietary tryptophane requirement and the importance of a desirable intestinal flora in this relationship are discussed.

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THE RELATION OF THE AMOUNT OF THIAMINE IN THE RATION OF THE HOG TO THE THIAMINE AND RIBOFLAVIN CONTENT OF THE TISSUE [1,2]

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TWO FIGURES

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Hughes ('41); Miller et al. ('43); Ensminger et al. ('43); and Ellis and Madsen ('44) have reported that the thiamine content of pork tissue is related to the amount of thiamine ingested by the pig. This means that pork may be enriched with extra thiamine by the use of feeds high in vitamin B_1. A program advocating the enrichment of pork by feeding swine rations high in this vitamin would increase the supply of thiamine in the American dietary.

Pork is one of the richest, naturally occurring sources of thiamine. It is eight times, and frequently higher in thiamine than beef according to values reported by Kemmerer and

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[2] This work was supported in part by grants from Armour and Company, Chicago and Spokane. The interest and cooperation of Col. E. N. Wentworth, Director, Livestock Bureau, Armour and Company, is gratefully acknowledged. The authors are indebted to Merck and Company, Rahway, N. J., for supplying gratis many of the crystalline vitamins used since the inception of this project. Statements of acknowledgment for products supplied by other companies are made as part of table 1. Acknowledgment is made for the help of Dr. C. L. Bedford, Assistant Horticulturist, in making the thiamine and riboflavin analyses. The cooperation in certain phases of this experiment by Miss M. A. McGregor, Assistant Chemist; Dr. W. M. Thorning, Assistant Veterinarian; and Dr. H. L. Eastlick, Associate Professor of Zoology, is gratefully acknowledged.

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Steenbock ('33); Waisman and Elvehjem ('41); and Richardson et al. ('44). According to values published by Waisman and Elvehjem ('41), pork is three to four times higher in thiamine than lamb. Therefore, if meat is to be enriched in thiamine, it follows that pork should logically be used since the pig is much more efficient than other meat animals in depositing the thiamine of the feed in its tissues.

The present investigation was undertaken in an effort to obtain additional information on the relationship of thiamine in the ration of the hog to the amount contained in the tissue. In addition, information was obtained on (1) the distribution of thiamine and riboflavin in various pork tissues, including the ham, loin, shoulder, liver, heart, and kidneys, (2) the efficiency of vitamin B_1 utilization, (3) the difference in the intestinal flora of pigs fed natural grain rations as compared to purified rations, (4) the changes in thiamine content of pigs of different ages by slaughtering animals at birth, at weaning, and at the end of the experimental feeding period, and (5) the changes in thiamine content of the hams of pigs obtained by performing biopsies on the biceps femoris at 3-week intervals during the growth period.

EXPERIMENTAL

Results obtained from preliminary feeding trials conducted in 1942 and 1943 were used as a basis for conducting these trials in the summer of 1944. The work reported herein confirms the results obtained in 1942 and 1943. In the 1944 trials, twenty pigs were divided into the five lots shown in table 1. The pigs were distributed equally in the several lots with respect to sex, weight, and age. The rations fed are shown in table 1.

A modification of the "paired-feeding technique" (Mitchell and Beadles, '30), was used in order to keep the feed intake constant among animals within the purified ration group (lots I, II, and III) and within the natural grain ration group (lots IV and V). Thus, in the purified ration lots, the feed consumption of the other nine pigs was limited to the amount consumed

TABLE 1

Rations fed to the five lots of pigs on the feeding trials.

GROUP	A			B	
LOT	I	II	III	IV	V
NO. OF PIGS	2	4	4	4	4
RATION TYPE	PURIFIED			NATURAL GRAIN	
Added thiamine or thiamine content of feed	None	0.19 mg. daily per kg. live weight	0.42 mg. daily per kg. live weight	0.11 mg. daily per kg. live weight (contained in feed)	0.21 mg. daily per kg. live weight (contained in feed)

The basic ration for all pigs in lots I, II, and III:

Purified ration:		Vitamin supplements (amounts daily per kg. live weight):		Wheat	46.0%	Wheat	36.0%
Casein [1]	26.1%	Riboflavin	0.12 mg.	Barley	35.0	Barley	35.0
Sucrose	57.7	Niacin	1.20 mg.	Tankage	13.5	Tankage	7.0
Lard	11.0	Pyridoxine	0.20 mg.	Alfalfa	5.0	Wheat germ meal	15.5
Salt mix [2]	5.2	Choline	10.00 mg.	Salt	0.5	Alfalfa	5.0
	100.0%	Calcium pantothenate	0.50 mg.		100.0%	Oyster shell	1.0
Plus 6.2% oat hulls treated to destroy thiamine [3] (added to supply bulk)		Shark liver oil [4]	167.00 mg.			Salt	0.5
		Viosterol	67.00 mg.				100.0%
		Vitamin E [5]	50.00 mg.				
		Vitamin K [5]	2.00 mg.				

[1] Purified casein obtained through cooperation of Mr. G. D. Turnbow, Vice President and General Manager, Golden State Co., Ltd., San Francisco, California.

[2] Salt mix:

Potassium chloride	6.7%	Calcium carbonate	16.9	Iron pyrophosphate	2.100%	Zinc oxide	0.016
Dicalcium phosphate	46.3	Magnesium carbonate	8.5	Manganese chloride	0.020	Cobalt carbonate	0.016
Sodium chloride	13.5	Potassium phosphate	5.7	Copper sulfate	0.020	Potassium iodide	0.227
							100.000%

[3] Acknowledgment is made of the cooperation extended by Mr. E. M. Williams, Manager, Livestock Chow Sales, Ralston Purina Company, St. Louis, Missouri, in supplying the oat hulls treated to destroy thiamine.

[4] Bio-Vita IOMA, Natural Shark Liver Oil Blend containing 10,000 units vitamin A per gram. Kindly supplied by Bioproducts, Inc., Astoria, Oregon.

[5] Supplied through courtesy of Lederle Laboratories, Pearl River, New York.

by the pig with the smallest appetite — with the quantity of thiamine being the only variable among the lots of pigs. The level of thiamine was controlled by adding definite amounts of the crystalline vitamin to the ration fed the pigs in lots II and III in the purified ration group. Likewise, the eight pigs fed the natural grain rations were considered as a group, with the feed intake of the other seven pigs limited by the pig consuming the least amount of feed. In these lots, the amount of thiamine was controlled by feeding natural grain rations composed of feeds containing different amounts of thiamine. All pigs were fed individually and promptly at three definite feeding periods daily — 6 A.M., 1 P.M., and 8 P.M.

The pigs were kept on raised floors in order to prevent coprophagy and were allowed outside as well as inside runs. The raised floors were removed and thoroughly washed three times daily as a further precaution to prevent the animals from consuming their feces.

In order to prevent the development of rancidity, the purified ration was kept in a refrigerator and not more than 2 days' feed requirements were mixed at one time. Refused feed was discarded after weighing and the individual troughs were cleaned and washed following each feeding.

The six B-complex vitamins fed (thiamine, riboflavin, niacin, pyridoxine, calcium pantothenate, and choline) were dissolved in 20% ethanol solutions and stored in a refrigerator. Vitamins A and D were kept separately, whereas E and K were mixed together. Two days' requirements of the B-complex vitamins and 3 days' requirements of the fat-soluble vitamins were fed every second and third day, respectively. The amounts were measured in calibrated pipettes and placed on a small amount of the feed on the top of the ration at feeding time. In this manner, struggling with the animals was avoided and complete consumption of the vitamins was obtained.

All the pigs were slaughtered when those on the thiamine deficient ration ceased to make any further nutritional progress. This point was reached when the pigs receiving no sup-

plement of thiamine lost their appetites and began to lose weight.

In order to provide a record of the thiamine content of the body tissues at various stages of development, two pigs of similar breeding to those fed on experiment were slaughtered at birth and two at weaning. In addition, one extra pig was fed in each of lots IV and V for the purpose of removing muscle sections from the biceps femoris at 3-week intervals during the experimental period. These samples were analyzed for thiamine content and used as a basis for comparison with samples from the pigs fed throughout the experimental period.

The comparative slaughter technique was employed in order to determine changes in thiamine content of several tissues from birth to weaning and from weaning to the end of the 56-day feeding period.

Following slaughter of the experimental animals, the carcasses were chilled for 24 hours at 33°F. Samples were taken within 24 to 48 hours following slaughter and were frozen immediately. They were stored at 0°F. for 30 days, during which time all the analyses were made. Ham, loin, shoulder, and kidney samples were taken from the right side of each carcass. After all extraneous fat was removed from these samples they were finely ground. The ground meat from each cut was thoroughly mixed before a sample was taken for assay. The left half of each carcass was skinned, boned, and ground together with the fat from that side, in order to secure a composite "sausage" sample.

Thiamine and riboflavin were determined by the thiochrome method, using the procedure developed by Conner and Straub ('41) for the combined determination of thiamine and riboflavin. The only modification of this method was in the manner of preparing the sample. This involved weighing an amount of the previously ground fresh or frozen sample and mixing in a Waring Blendor with the desired volume of extractant. Aliquots were obtained and transferred to volumetric flasks.

Samples for the determination of fat and moisture were removed from the bottles of frozen meat and weighed at the

same time as those taken for the vitamin analyses. The method described by Pitman ('32) was used to determine moisture and fat in the "sausage" samples, whereas slight modifications were made of the A.O.A.C. ('40) method for the determination of fat and moisture in the other samples. The drying time for the moisture determination was extended an additional 3 hours on all samples. The extraction of fat from the liver samples was extended to 48 hours.

EXPERIMENTAL RESULTS AND DISCUSSION

A record of the feed consumption and body weight gains is given in table 2. Some differences were observed among lots of pigs in average daily gains made over the 8-week feed-

TABLE 2

Record of feed consumed and gains in weight by the pigs on the feeding trials.

Group	A			B	
Lot	I	II	III	IV	V
	PURIFIED			NATURAL GRAIN	
Ration	No thiamine	0.19 mg. thiamine daily per kg. live weight	0.42 mg. thiamine daily per kg. live weight	0.11 mg. thiamine daily per kg. live weight (contained in feed)	0.21 mg. thiamine daily per kg. live weight (contained in feed)
Number of pigs	2	4	4	4	4
Length of feeding period (days)	56	56	56	56	56
Average initial weight (pounds)	41.5	41.8	41.7	45.2	43.5
Average final weight (pounds)	70.5	80.2	80.5	78.8	84.0
Average total gain (pounds)	29.0	38.4	38.8	34.6	40.5
Average daily gain (pounds)	0.52	0.68	0.69	0.62	0.72
Average daily ration (pounds)	1.77	1.77	1.77	2.16	2.16
Feed per cwt. gain (pounds)	341	258	255	349	298

ing period. The pigs fed the thiamine deficient ration gained at a slower rate than the pigs fed 0.19 mg. thiamine daily per kilogram body weight. Increasing the thiamine intake from 0.19 to 0.42 mg. daily per kilogram body weight had no significant effect on the average daily gains or efficiency of feed utilization. Similar differences in body weight gains were noted between lots IV and V. Since the ration fed in lot V included an additional ingredient (wheat germ meal) as compared with the ration fed in lot IV, the increased gains cannot be attributed alone to the additional thiamine in the ration because the wheat germ meal may have contributed other factors which may have influenced body gains and efficiency of feed utilization.

Of much importance is the finding that the pigs on the thiamine deficient ration required 83 to 86 lbs. more feed, respectively, per 100 lbs. gain than the pigs in lots II and III which were fed the same ration plus different levels of thiamine. Since the pigs on the thiamine deficient ration ate the same amount of feed as the pigs in lots II and III, and still required much more feed per 100 lbs. gain, this definitely shows that thiamine is concerned with the efficiency of feed utilization. This is very logical since thiamine is involved in carbohydrate metabolism. Further evidence to demonstrate the increased efficiency of feed utilization by the pigs fed either the purified ration plus thiamine or the natural grain ration is shown by the percentages of body fat in the animals of each lot. The carcasses of the pigs receiving the purified ration without thiamine contained only 21% fat. The carcasses of those receiving either the purified ration plus thiamine or the natural grain ration all contained between 25 and 27% fat.

Essentially the same type of symptoms as those recorded by Van Etten et al. ('40) and Ellis and Madsen ('44) resulted from the feeding of the thiamine deficient ration. Vomiting was the first symptom observed in these pigs fed a thiamine deficient ration. It was first observed and occurred frequently after the animals had been on the thiamine deficient ration for 33 days. Appetite started to decrease during the latter

TABLE 3

Average thiamine content of tissues[1] *(fresh, and dry, fat-free bases) from pigs fed different levels of thiamine.*

LOT	RATION	NO. OF PIGS	BASIS OF THIAMINE CONTENT	SAUSAGE	HAM	LOIN	SHOULDER	HEART	LIVER	KIDNEY
I	Purified no thiamine added	2	Fresh	.24	1.64	1.28	.52	.79	.86	.89
			Dry, fat-free	1.43	7.33	5.56	2.41	4.12	3.48	4.30
II	Purified 0.19 mg. thiamine daily per kg. body weight	4	Fresh	3.66	8.54	8.74	7.29	5.58	4.95	4.73
			Dry, fat-free	29.17	38.34	38.86	32.10	28.86	18.17	26.17
III	Purified 0.42 mg. thiamine daily per kg. body weight	4	Fresh	5.64	10.65	11.34	9.38	6.11	4.37	5.26
			Dry, fat-free	37.73	41.32	49.42	41.08	30.79	16.32	33.24
IV	Natural Grain 0.11 mg. thiamine daily per kg. body weight (contained in feed)	4	Fresh	4.55	10.36	10.29	7.07	4.58	4.03	3.20
			Dry, fat-free	31.29	45.23	43.12	32.98	24.77	15.06	17.71
V	Natural Grain 0.21 mg. thiamine daily per kg. body weight (contained in feed)	4	Fresh	5.67	11.73	12.69	6.76	4.76	4.03	4.00
			Dry, fat-free	36.59	51.86	52.46	43.66	26.53	14.48	22.59

[1]Micrograms per gram.

half of the experimental feeding period, and was followed closely by a corresponding retardation of growth. Ellis and Madsen ('44) observed that failing appetite usually was the first sign of thiamine deprivation. In the 1943 and 1944 work at this station, vomiting occurred and was followed in a day or two by a decreased appetite. A slight staggering gait was noted in the thiamine deficient pigs during the final 5 days of these trials. On the fifty-third day one of these pigs showed signs of muscular incoordination when it trembled and fell

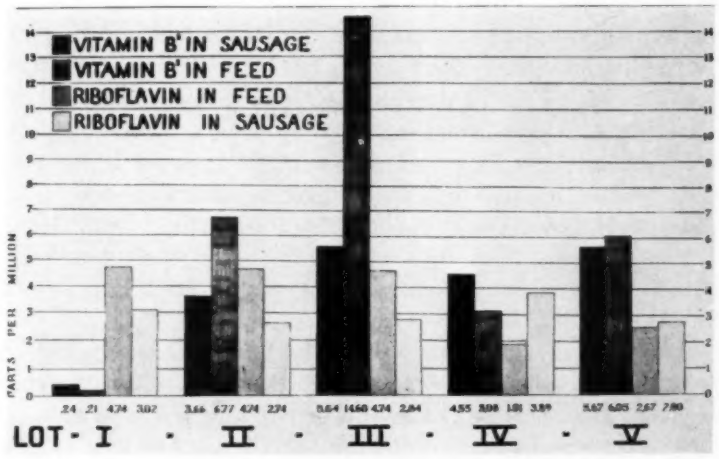
VITAMIN B' IN SAUSAGE
VITAMIN B' IN FEED
RIBOFLAVIN IN FEED
RIBOFLAVIN IN SAUSAGE
PARTS PER MILLION
.24 .21 4.74 3.02
3.66 6.77 4.74 2.74
5.64 14.60 4.74 2.84
4.55 3.08 1.81 3.89
5.67 6.05 2.67 7.80
LOT - I - II - III - IV - V

Fig. 1 The relationship of thiamine and riboflavin content of swine rations to the amount of these vitamins deposited in the pork tissues.

while eating. After several unsuccessful attempts to regain a standing position, the pig was finally able to stand without further indications of muscular incoordination.

Data showing the average thiamine content of the pork are presented in table 3 and figure 1. The values in table 3 show that the ham and loin have a consistently higher level of thiamine than the other tissues. Generally, the order of the tissues in thiamine content from highest to lowest is as follows: loin and ham, shoulder, heart, liver, and kidney. The thiamine content

of the composite carcass sample ("sausage") made from the left half of each carcass is intermediate between the level of thiamine in the heart and liver. The thiamine content of the "sausage" is a good indication of the relative level of this vitamin in the ration (fig. 1).

The thiamine values shown in table 3 indicate that generally the pigs fed natural grain rations were much more efficient in depositing the thiamine in the ration in their tissues. For example, ham samples from pigs in lots III and IV, show that the pigs in lot IV fed the natural grain ration received about one-fourth as much vitamin B_1 as those fed the purified ration in lot III. Yet, the pigs in lot IV had about as much thiamine deposited in their hams as those pigs fed approximately four times as much thiamine in lot III (purified ration). This difference in favor of natural grain rations, might possibly be due to the fact that thiamine in natural grains is released more slowly and is therefore more efficiently utilized. The manner in which the synthetic thiamine was fed and the ease of excretion of the excess vitamin may have contributed to less efficiency of utilization.

Our findings with respect to the higher thiamine content of pork skeletal muscle as compared to liver coincide with those of Miller et al. ('43). The liver is apparently the least responsive to the effect of added thiamine in the ration. The kidney and heart are more responsive than the liver to added dietary thiamine, but not nearly as much as the skeletal muscles.

Statistically, the difference between ration groups shown in table 3 was highly significant on both the fresh and dry, fat-free bases. However, the greater portion of the difference was due to the low values obtained for the pigs in lot I, which were fed the thiamine deficient ration. On the other hand, the variation among pigs within lots was large, indicating that great variability exists among pigs given the same treatment. As Miller et al. ('43) have indicated, this is further evidence to explain some of the wide variations in thiamine values for pork which have been reported from time to time.

Using the comparative-slaughter technique, it was shown that the thiamine content of pork, on a fresh basis, increased from birth to weaning and decreased gradually from weaning to the end of the 56-day feeding period. Data showing these values are presented in table 4. No explanation is known for the marked increase in thiamine from birth to weaning. A possible explanation might be that the pig is receiving a high fat diet (on a dry-matter basis) while suckling its mother, and as a result needs less thiamine and hence deposits more in the tissue. Ellis and Madsen ('44) have shown that fat lessens the pig's need for thiamine.

TABLE 4

Changes in thiamine content (fresh basis) from birth to weaning, and weaning to the end of the 56-day feeding period in six pork cuts.

Pigs	Tissue sample					
	Ham	Loin	Shoulder	Kidney	Heart	Liver
Birth (2 pigs)	5.65	8.30	9.42	5.52	8.84	5.98
Weaning (2 pigs)	20.17	18.62	16.02	9.52	14.82	17.02
After 56-day feeding period (ave. experimental pigs)	8.58	8.87	6.20	3.61	4.36	3.65

[1] Micrograms per gram.

Observations in this study indicate that true storage of thiamine does occur in the pig and that the pig uses stored thiamine. At the beginning of the experimental feeding period, the analyses of two animals representative of those used in the experiment, showed that each pig contained approximately 200 mg. of thiamine in its entire carcass (exclusive of the usual offal) at the start of the experiment. At the end of the 56-day feeding period, those animals fed the thiamine deficient ration still contained slightly over 77 mg. of thiamine in their entire carcass (exclusive of offal). Since the purified ration supplied approximately 0.16 mg. of thiamine daily to each of these animals, it is reasonable to assume

that these animals were using stored thiamine at the rate of 2.21 mg. per animal daily, or 0.09 mg. daily per kilogram of body weight. Certainly there was no dietary supply of thiamine which could have supplied these animals with the 77 mg. of thiamine which remained in their bodies at the end of the 56-day depletion period. Another reason for stating that storage of thiamine occurs and that the pig can utilize this stored thiamine for long periods of time, is the fact that it took 33 days before the appetites of these animals were affected and 56 days before their appetites were lost completely.

TABLE 5

Changes in thiamine content of ham (biceps femoris muscle) during the suckling and early feeding period of pigs.

AGE	THIAMINE CONTENT OF HAM (FRESH BASIS)[1]
At birth (2 pigs)	5.65
At weaning (2 pigs)	20.17
At start of feeding period (2 pigs on grain ration)[2]	9.97
After 23 days of feeding (2 pigs on grain ration)[2]	7.48
After 44 days of feeding (2 pigs on grain ration)[2]	6.50

[1] Micrograms per gram.

[2] Determined on same two pigs by performing biopsies.

Muscle sections of the hams of live pigs taken [4] at 3-week intervals and analyzed for thiamine show that the thiamine content of the ham (biceps femoris) increased from birth to weaning and gradually decreased after weaning to the forty-fourth day of the experiment period, at which time the last ham sample was taken. Changes in water and fat content of the tissues from birth to weaning and later stages may account for a part of the change in thiamine content during this period. However, it is believed that the greater portion of the change in thiamine content of the ham muscle may be attributed to other causes. These data, presented in tables 4 and 5, were obtained from assays made of ham tissues from two new-born

[4] Obtained by Dr. P. J. Pfarr, Assistant Professor of Veterinary Anatomy, and Dr. E. C. McCulloch, Research Veterinarian.

pigs, two pigs at weaning time, and the two extra pigs on the feeding experiments from which live ham muscle (biceps femoris) samples were taken at 3-week intervals. Samples were taken alternately from the left and right leg so that there was a 6-week interval before a second biopsy was performed on the same leg. These biopsies were performed three times with no apparent harmful effect or interference with the locomotion of the two pigs.

Average riboflavin values for the tissues from the pigs slaughtered at the termination of the experiment are shown in table 6. All lots of pigs fed the purified ration received the same level of riboflavin whereas the two lots of pigs fed the natural grain rations received slightly differing amounts of riboflavin. However, no significant difference was noticed in riboflavin deposition among lots of pigs, indicating that the dietary level of riboflavin has much less effect on skeletal muscle tissue content of this vitamin than is true in the case of thiamine. Also, pork skeletal muscle evidently contains much less riboflavin than thiamine. However, pork heart, liver, and kidney contain more riboflavin than the skeletal muscles and more riboflavin than thiamine.

The riboflavin values shown in table 6 indicate that pigs fed natural grain rations were more efficient in depositing the riboflavin in the ration in their tissues. For example, the composite carcass samples from pigs in lots III and IV, show that the pigs in lot IV, fed the natural grain ration, received $\frac{1}{2}$ as much riboflavin as those fed the purified ration in lot III. Yet, the pigs in lot IV had even higher levels of riboflavin deposited in the sausage than those pigs fed the purified ration with higher levels of riboflavin.

Results of this work indicate that a thiamine deficient ration fed to pigs has some effect on increased riboflavin deposition in the heart and liver. Data in table 6 show that the hearts and livers of the pigs fed the thiamine deficient ration contained slightly more riboflavin than the hearts and livers of pigs fed adequate levels of thiamine. This finding is in agreement with those of Singher et al. ('44) and Luecke et al. ('44),

TABLE 6

Average riboflavin content of tissues (on fresh and on dry, fat-free bases) from pigs on different levels of thiamine and riboflavin intake. All values are micrograms per gram of tissue.

LOT	RATION	NO. OF PIGS	BASIS OF THIAMINE CONTENT	SAUSAGE	HAM	LOIN	SHOULDER	HEART	LIVER	KIDNEY
I	No thiamine; 0.12 mg. riboflavin daily per kg. body weight	2	Fresh	3.02	3.68	3.00	3.62	14.73	23.88	16.49
			Dry, fat-free	18.38	16.36	12.70	16.72	76.62	96.26	80.81
II	0.19 mg. thiamine daily per kg. body weight; 0.12 mg. riboflavin daily per kg. body weight	4	Fresh	2.73	4.54	3.32	4.19	13.41	22.88	10.83
			Dry, fat-free	17.65	20.69	14.82	18.69	69.48	85.45	60.98
III	0.42 mg. thiamine daily per kg. body weight; 0.12 mg. riboflavin daily per kg. body weight	4	Fresh	2.84	3.35	2.61	2.74	14.22	23.67	15.91
			Dry, fat-free	18.97	15.28	11.36	12.03	71.71	88.02	86.47
IV	0.11 mg. thiamine daily per kg. body weight; 0.06 mg. riboflavin daily per kg. body weight (contained in feed)	4	Fresh	3.89	2.81	2.41	2.57	11.45	22.27	15.37
			Dry, fat-free	19.45	12.98	10.37	12.00	61.86	82.97	85.17
V	0.21 mg. thiamine daily per kg. body weight; 0.09 mg. riboflavin daily per kg. body weight (contained in feed)	4	Fresh	2.80	3.07	2.93	2.96	11.79	19.76	19.04
			Dry, fat-free	17.91	13.63	12.20	13.26	65.74	71.20	105.56

who reported that a thiamine deficiency results in an increased riboflavin concentration in the liver of rats. A possible explanation for the increased riboflavin concentration in the liver and heart of the pig may be that with a thiamine deficiency there is a slowing of body metabolism and as a result less riboflavin is needed. Hence, the riboflavin accumulates in the liver and heart which are the main storage organs for this vitamin.

Observations were made of the rate of heartbeat, rate of respiration, and rectal temperature throughout the experiment. A slowing of the heartbeat and respiratory rate in the thiamine-deficient animals was noted during the last week preceding the end of the experiment. Rectal temperatures of these animals dropped during the last 4 days to slightly below 99°F. Upon slaughtering, enlarged hearts were found in the animals fed the purified, thiamine deficient ration. The hearts of the two thiamine deficient pigs were 54 and 91% heavier than the average of the hearts of the other pigs on this experiment. Pictures of representative hearts of a thiamine deficient pig as compared to the heart of one of the pigs which received an adequate level of thiamine are shown in figure 2.

The bactericidal action of the blood sera of the pigs against Escherichia coli, Salmonella typhimurium, Eberthella typhosa, and Staphylococcus aureus did not appear to be influenced by type of diet or thiamine level. Coliform counts of the feces, on a wet basis, of the pigs receiving purified rations, either without thiamine or at different levels of thiamine, were approximately 1000 times higher than the coliform counts, very largely Escherichia coli, of the feces of pigs receiving natural grain rations. This suggests a possible difference in intestinal vitamin synthesis between pigs fed a purified as compared to a natural grain ration, since Gant et al. ('43) obtained indications that Escherichia coli is the organism mainly responsible for the synthesis of biotin and folic acid or related factors. Work by other investigators substantiates this possibility. Cunha et al. ('43) demonstrated that rats on a basal ration composed largely of corn (natural grain ration) re-

ceived an insufficient supply of the norite eluate fraction (a source of folic acid, vitamins B_{10} and B_{11}). Such a deficiency can ordinarily be produced with rats receiving a purified ration only when a sulfa drug is added, as was demonstrated by Black et al. ('41). This indicates that corn, or other constituents of a natural grain ration, in some manner, decreases intestinal synthesis of certain vitamins. This has recently

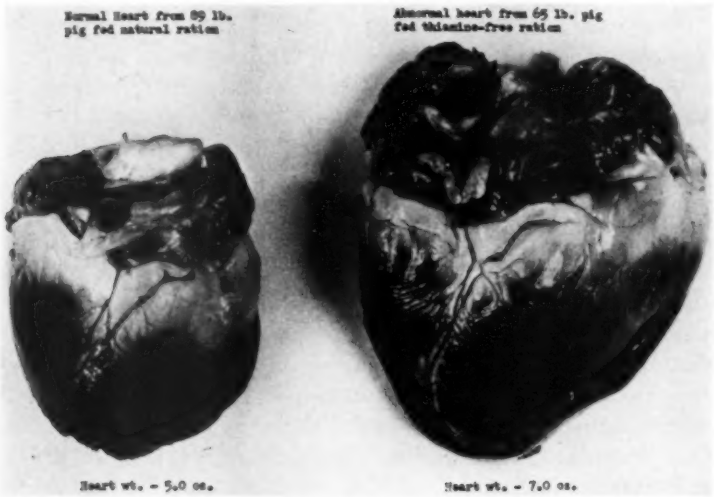
Normal heart from 89 lb. pig fed natural ration
Abnormal heart from 65 lb. pig fed thiamine-free ration
Heart wt. - 5.0 oz.
Heart wt. - 7.0 oz.

Fig. 2 The heart at left is from a pig fed the purified ration with adequate thiamine. The heart at the right is that of a pig fed the same ration without thiamine. Live weights of the two pigs were 89 and 65 lbs., respectively.

been confirmed with the chick and rat. Briggs et al. ('45) showed that when corn meal was substituted for part of the dextrin in a purified ration, growth, mortality, and hemoglobin formation of chicks on the basal ration was poorer. Krehl et al. ('45) showed that a growth retardation was caused in rats when 40% of corn grits was included in a low protein ration. All of this work strongly indicates that there may be a difference in intestinal vitamin synthesis by animals fed natural grain rations as compared to purified rations.

SUMMARY AND CONCLUSIONS

The poorest gains were made by pigs on the thiamine deficient ration. Likewise, the pigs on the thiamine deficient ration required much more feed per pound of gain, definitely showing that thiamine is concerned with the efficiency of feed utilization by the pig.

Efficiency of utilization of the thiamine and riboflavin in the ration was much greater for pigs fed the natural grain rations than for those fed the purified rations.

A positive relationship exists between thiamine intake and the deposition of this vitamin in the tissues of hogs. This makes it possible to increase the content of thiamine in pork by using feeds high in this vitamin. Usually the loin and ham are highest in thiamine content, followed by the other cuts in the following order: shoulder, heart, liver, and kidney.

Pork liver is higher in riboflavin content than the other pork cuts, followed by the kidney, heart, ham, shoulder, and loin. A thiamine deficiency had no lowering effect on the deposition of riboflavin in the skeletal muscle tissues. There was a slight increased concentration of riboflavin in the liver and heart of the pigs on a thiamine deficient ration as compared to the pigs on a ration containing adequate thiamine.

Symptoms observed on the thiamine deficient pigs were vomiting, anorexia, slight staggering, cyanosis, and a reduction in rectal temperature, heart beat and respiration during the final stages of the experiment. Enlarged hearts were obtained from the pigs on the thiamine deficient ration.

Coliform counts of the feces of pigs fed the purified ration, with or without thiamine, were approximately 1000 times higher than for pigs fed natural grain rations. This may suggest a possible difference in intestinal vitamin synthesis between pigs fed natural grain rations as compared to purified rations. Further evidence for this possibility is presented and discussed.

Data were obtained to show that storage of thiamine occurs and that the pig can utilize stored thiamine over a long

period of time. Evidence for this is that 56 days were required for the pigs to lose their appetites after being placed on a thiamine deficient ration.

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FURTHER STUDIES ON THE CALCIUM REQUIREMENT OF PRESCHOOL CHILDREN [1, 2]

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TWO FIGURES

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INTRODUCTION

In 1939 this laboratory published a report dealing with the calcium requirements of five girls, from 3 to 5 years old (Outhouse, Kinsman, Sheldon, Twomey, Smith and Mitchell, '39). At that time the authors stated that these well-nourished children needed less than 1 quart of milk daily for the satisfaction of their calcium needs. The present paper, dealing with a similar investigation of preschool boys, presents a similar conclusion; only one of the seven subjects needed as much as 1 pint of milk daily and he could have satisfied his individual requirement for calcium by ingesting, in addition to an otherwise satisfactory diet, 720 gm. of milk daily.

EXPERIMENTAL

The children used in this study were seven normal, healthy boys ranging in age from 2.7 to 5.8 years at the beginning of the experiment. A description of their height-weight-age

[1] A preliminary report of these data was made before the Amercain Home Economics Association at its annual meeting in Pittsburgh, June, 1939.

[2] Aided by a grant from the American Dry Milk Institute, Inc., Chicago.

relationships in comparison with the standards of Grandprey ('33) has been presented in a former publication (Kinsman, Sheldon, Jensen, Bernds, Outhouse and Mitchell, '39). Four of the subjects (R, C, Br and Jw) were presumably well nourished in respect to calcium since they had been accustomed not only to drinking liberal quantities of milk at each meal but also to having for one meal each day either bread and milk or a cream soup. The dietary history of the other three boys was unknown. Subject D was brought from another children's home expressly for the experiment and W and G, brothers, because of unsatisfactory home care were admitted to the Cunningham Children's Home on the day on which the experiment started.

A description of the experimental conditions under which the subjects were held, as well as the analytical methods used, may be found in the publication of Kinsman et al. ('39) and of Outhouse et al. ('39). Only the important features of the regimen need be repeated here: (a) a daily allowance of approximately 1000 international units of vitamin D was fed as cod-liver oil; (b) the low-calcium content (i.e., 339 mg. daily) of the basal dietary was purposely achieved by limiting the amounts of vegetables to only one or two servings daily and the quantity of milk solids to the equivalent of approximately 200 gm. of fluid milk daily; (c) the basal dietary which was fed throughout the study was supplemented during periods I, III, IV and V by non-fat dry milk solids [3] which brought the average intakes of calcium to 1600, 555, 704, and 904 mg., respectively, during these periods; (d) the metabolic periods were 7 days in length and period I to V, respectively, lasted 91,[4] 49, 35, 28 and 63 days.

The method for computing the calcium requirement makes use not only of data for calcium retentions during periods I

[3] The calcium content of the non-fat dry milk solids ranged from 12.3 to 13.5 mg. per gram. (In two former publications, an error in the decimal place was made in reporting the calcium content of the milk solids.)

[4] Subject D was kept at this high level for 126 days.

to V [5] but also of the previously reported data (Kinsman et al., '39) concerning the extent to which each subject utilized the calcium of milk. The retentions of calcium during the period of generous calcium intake, i.e., period I, were compared with the balances during periods II, III, IV and V to determine if like retentions had been observed at lower levels of intake. In those instances in which it was reasonable to suspect that equally high retentions of calcium might have been observed at a significantly lower level of intake, the calcium requirement was obtained by use of the following formula:

$$\frac{\text{Maximum calcium retention in mg.}}{\text{Per cent utilization of milk calcium}} \times 100 = \text{Calcium requirement in mg.}$$

RESULTS

The skeletal growth of the subjects is shown in figure 1 in comparison with the growth of boys from families above the average in economic and educational status (Simmons, '44). Five of the boys had horizontal lengths shorter than the mean for Simmons' group. However, in relation to the mean rate of growth of the latter, the rates of growth of the 7 subjects during the total experimental period, from 7 to 18 months for the various subjects, were as follows: that of subject D was considerably faster whereas those of R, W, G and Jw were parallel, and those of C and Br were probably somewhat slower. For the period under discussion in this paper; i.e., from the fifth [6] to the thirteenth week, the growth in length of the body was satisfactory for all subjects with the exception

[5] Detailed data concerning the weekly intake and the urinary and fecal excretions of calcium for these seven boys and for the five girls previously studied will appear in a forthcoming publication by I. G. Macy entitled "Nutrition and Chemical Growth in Childhood," vol. II. Original Data. Charles Thomas Press, Springfield, Ill.

[6] The first measurements on all subjects except D were taken on the thirty-fourth day of the experiment; hence that portion of the curves referring to period I is represented by the first 45 days. D's measurements were taken at the beginning of the study.

of W whose increment during this short period only appeared to be smaller than the mean for children of his age.[7]

The calcium retentions of the subjects during the period when they were receiving 1600 mg. of calcium daily are recorded in figure 2 in terms of the cumulated retentions. In the lower set of curves data are given for the entire period. Considerable variability in the week-to-week balances of a given

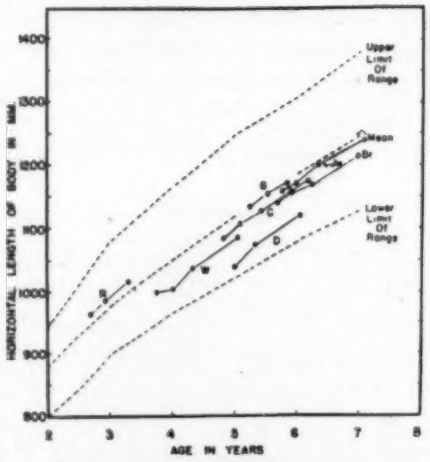

Fig. 1 The growth in horizontal length of the seven boys, R, W, C, D, G, Jw and Br, is shown here in comparison with the mean and the upper and lower limits of range of similar measurements for a group of boys from families above the average in economic and educational status (Simmons, '44). The first measurements were taken on the thirty-fourth day of the experiment for all subjects except D, whose measurements were taken at the beginning of the study.

subject is evident. From a cursory inspection of the curves one might conclude that the subjects differed markedly in the rate at which they stored calcium. Actually, however, four of the subjects, i.e., W, G, C and Jw, accumulated approximately the same quantity of calcium during the 12- to 13-week period. Moreover, during certain consecutive weeks the other

[7] The increment in acromial breadth for this period likewise was small; those for cristal and trochanteric breadth, however, were greater than the mean observed in Simmons' group.

three subjects stored calcium at the same rate as did the former four subjects. The uniformity in the accretion of calcium is clearly shown in the upper set of simplified curves [8] constructed from data for selected weeks, i.e., the entire period

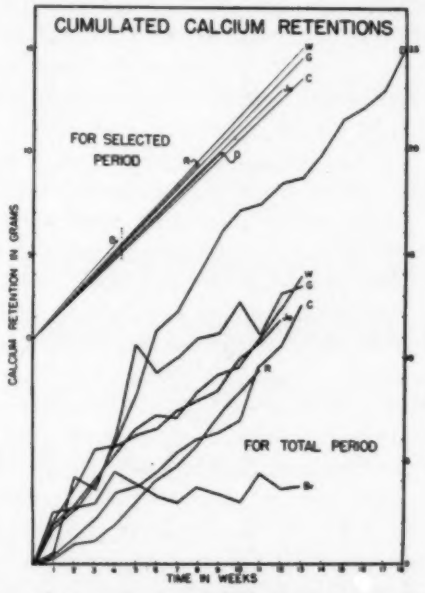

Fig. 2 The cumulated calcium retentions of the seven boys are shown (a) weekly for the entire experimental period in the lower set of curves and (b) for selected weeks in the upper set of curves which have been constructed by drawing a straight line between the zero point and the point for the total cumulated retention. The black dots recorded at 30 days in the upper set of curves refer to the cumulated calcium retentions of eight well-nourished children during the last month of a 6-month study conducted by Macy ('42).

for W, G, C and Jw, the last 9 weeks for D, the last 8 for R and the first 4 weeks for Br. For these selected weeks the daily retentions averaged 150, 154, 137, 143, 148, 161 and 143 mg., respectively, for subjects R, W, C, D, G, Br and Jw (table 1).

[8] In order to avoid the confusion introduced by the variable weekly balances a straight line has been drawn between the zero point and the point for total retention for each subject.

TABLE 1

Daily calcium-requirement values and data upon which they are based.

SUBJECTS				CALCIUM RETENTION DURING PERIOD I					CALCIUM DATA DURING PERIODS OF LOWER INTAKE				PROBABLE CALCIUM REQUIREMENT			PROPOSED MILK REQUIREMENT [2]
Name	Description [1]			For entire period	For selected weeks				Period	Intake	Retention	Utilization	Total	per kg.	per cm.	
	Age	Weight	Height		No. of weeks	total	per kg.	per cm.								
	yr-mo	*kg.*	*cm.*	*mg.*		*mg.*	*mg.*	*mg.*		*mg.*	*mg.*	%	*mg.*	*mg.*	*mg.*	*gm.*
									II	328	63					
R	2– 8	16.0	96.5	116	8	150	9.4	1.55	III	554	94	17.8	843	53	8.7	452
									IV	692	143					
									V	891	158					
W	3– 9	16.4	98.9	154	13	154	9.3	1.55	II	329	68	21.3	723	44	7.3	352
									III	548	116					
									IV	703	145					
									V	899	183					
									II	347	63					
C	4–10	20.3	108.5	137	13	137	6.7	1.26	III	560	81	20.0	685	35	6.6	321
									IV	712	136					
									V	909	145					
									II	350	54					
D	5– 0	16.6	103.8	197	9	143	8.6	1.36	III	504	96	17.2 [3]	831	50	8.0	442
									II	343	53					
G	5– 3	20.4	113.2	148	13	148	7.2	1.30	III	567	83	12.7	1165	57	10.3	720
									IV	728	98					
									V	915	126					
									II	344	60					
Br	5– 8	22.5	114.0	42	4	161	7.1	1.41	III	556	106	19.5	826	37	7.2	438
									IV	699	119					
									V	904	179					
									II	345	64					
Jw	5– 9	20.1	116.0	143	12	143	7.1	1.23	III	545	119	17.8 [4]	803	40	6.9	419
									IV	697	110					
									V	905	135					
Ave.				134		151	7.9	1.38					839	45	7.8	478

[1] Data collected at the fifth week of the study.

[2] Calculated on the basis of the difference between the total requirement and that quantity (i.e., 300 mg. as an average) which preschool children could be expected to obtain from the non-milk foods of their dietary.

[3] Satisfactory data for the computation of per cent utilization were not obtained for this subject; hence this value refers to percentage retention.

[4] This value, the mean of the percentage utilization data derived from 3 periods, is used here rather than the originally-reported value of 27.5 based on data for one period. This value, 17.8%, more closely agrees with the mean value for percentage retention during 4 periods; i.e., 17.7%. Moreover this subject's utilization of the calcium in $CaHPO_4$ was only 17.3%.

The mean retention for the group was 151 mg. a value somewhat higher than the average retention of 134 mg. observed for the total, non-selected periods. On the basis of body weight and skeletal length, respectively, the above retentions are 9.4, 9.3, 6.7, 8.6, 7.2, 7.1 and 7.1 mg. per kilogram and 1.55, 1.55, 1.26, 1.36, 1.30, 1.41 and 1.23 mg. per centimeter for the children in the above sequence.

Table 1 also presents the calcium balances during subsequent periods of reduced calcium intake. In every instance the retentions of calcium were greater during period V than during period IV. This fact alone, however, should not be taken as evidence that the daily requirement for dietary calcium for all the subjects more nearly approximated 900 mg. than 700 mg. This should be obvious when it is realized that the low levels of calcium during some of the periods may not have been sufficient to allow for normal calcification of the skeletal tissues formed during that time and that consequently a portion of the calcium stored during subsequent periods of excess dietary calcium may have been used to bring those previously formed bones to a state of physiological saturation; hence the retentions during period V are not necessarily representative of the well-nourished child.

The computed requirements for dietary calcium are also given in table 1. None of the children needed 1600 mg. of calcium daily. Subject G, because of his exceedingly poor capacity to utilize calcium, apparently required as much as 1165 mg.; i.e., $\frac{148}{12.7\%} \times 100$. Subject R's retention at the 891 mg. level was similar to that during period I; a requirement of 843 mg. seems probable. In placing Br's requirement at less than 900 mg., it is pertinent to mention the long period of possibly inadequate calcification; i.e., 9 weeks in period I during which very poor utilization was observed even though the daily intake approximated 1600 mg., in addition to the 16 weeks of periods II through IV; if 161 mg. represents his maximal retention of calcium under good nutritive conditions, a daily intake of 826 mg. should be sufficient for him. The requirements of D and Jw, respectively, are placed at 831 and

[4] This value, the mean of the percentage utilization data derived from 3 periods, is based on data for one period. This value, 17.8%, more closely agrees with the mean value for percentage retention during 4 periods; i.e., 17.7%. Moreover this subject's utilization of the calcium in $CaHPO_4$ was only 17.3%.

803 mg. solely on the basis of their retentions during period I and their probable ability to utilize calcium; unfortunately the retentions of these two subjects during periods of reduced calcium intakes showed no consistent proportionality to the intake and hence do not provide contributory evidence. Subjects W and C apparently needed a smaller quantity of calcium than did the other subjects; at intakes approximating 700 mg. their calcium retentions were similar to those during period I, hence their requirement can be placed, with reasonable assurance, at 723 and 685 mg., respectively. The mean of these proposed values for requirement for dietary calcium; i.e., 843, 723, 685, 831, 1165, 826 and 803, respectively, for subjects R, W, C, D, G, Br and Jw, is 839 mg. On the basis of weight these values become 53, 44, 35, 50, 57, 37 and 40 mg. per kilogram, whereas the percentimeter requirements are 8.7, 7.3, 6.6, 8.0, 10.3, 7.2 and 6.9 mg., listed in the same order as the values given above; the mean values for the two sets of data are 45 mg. and 7.8 mg., respectively.

DISCUSSION

The method used in this experiment for assessing the calcium requirements of the preschool boys involves the determination of the calcium retention during a period of generous calcium intake and its conversion into terms of dietary calcium, using data concerning the extent to which each subject could utilize milk calcium. Inherent in this procedure is the assumption that the child's only requirement for calcium is for growth; if he needed to ingest calcium to replace endogenous losses, then the total requirement would be greater than the values computed by this method. However, our investigations on children, including six of those in this study, have indicated that, at levels of intake not exceeding the requirement, no extra calcium need be provided for maintenance purposes (Kinsman et al., '39). If this is true, then the approach used in this study is not only theoretically sound but also serves as a means of determining fairly precise calcium requirements.

An important feature of this study was the feeding of generous quantities of calcium at the beginning of the study in an attempt to insure physiological calcification of previously formed, but possibly inadequately calcified bone in order to obtain subsequently, under conditions of generous calcium feeding, an accurate measure of the calcium required for growth. Some of the subjects; i.e., R, C, Br and Jw, as previously mentioned, should have been in a satisfactory state of calcium nutrition at the beginning of the study and hence their retentions during the entire 13 weeks of period I should be indicative of their day-to-day requirements for calcium. Nevertheless a selection of data was made in the case of three of these children. Subject R, following the first week of the experiment, was ill for 2 weeks; since his calcium balances during the first 3 weeks following the illness were not typical of those during the subsequent 8 weeks, they were discarded. Subject Br was virtually in calcium equilibrium during the last 9 weeks of the experiment, a response not observed in this subject even when his daily ingestion of calcium was markedly reduced as in period II. Therefore the conclusion was reached that the child's needs might be more accurately expressed by the calcium retentions during the first 4 weeks than by the mean retention of the entire 13 weeks; hence the data for the last 9 weeks were discarded. The retentions of two of the children were greater during the early weeks of the experiment than they were during the latter part, suggesting a partial inadequacy of the calcium reserves at the beginning of the experiment. In the case of D whose daily retention of calcium averaged 253 mg. during the first 9 weeks but only 143 mg. during the second 9 weeks, the data for the first 9 weeks were discarded. In the case of subject G, who retained 221 mg., as an average, during the first 6 weeks and 84 mg. during the last 7 weeks, the data for the entire 13 weeks were averaged. However, if this child was undernourished at the beginning of the experiment, the latter figure, i.e., 84 mg., doubtless would be more representative of his true requirement than would the mean value for the entire

period, namely, 147 mg. Whether or not such a selection of data as employed in the case of subjects R, D and Br is defensible is open to argument. Two results, however, have already been mentioned; namely, a greater uniformity in calcium retention among the children and a higher mean value for the group. In a group of eight children receiving 900 mg. of calcium daily for 6 months Macy ('42) observed a progressive increase in uniformity of, and in mean value for, the calcium retentions. For purposes of comparison the cumulations of calcium by her subjects during the last month of the experiment are recorded as black dots; i.e., at 30 days, in the upper set of curves in figure 2. It is obvious that the uniformity in calcium retentions, as expressed by the values assigned to the children in the present study, is as great as that observed for the children in Macy's study after 5 months on a calcium-adequate regimen.

The seven subjects in this study bring to a total of twelve the number of preschool children whose calcium metabolism has been studied in this laboratory. The daily retentions proposed for the boys average 151 mg. whereas those for the girls average 128 mg., values which, when converted to a per-kilogram basis, agree remarkably well; i.e., 7.9 and 7.8 for the boys and girls, respectively. Similar mean values can be found in the literature for preschool children who have been held on experimental diets for considerable time; i.e., 8.0 mg. per kilogram for Porter-Levin's ('32–'33, '33–'34) five children, 7.6 mg. for the five of Hawks et al. ('42), 8.1 mg. for the four reported by both Bonner et al. ('38) and Hummel et al. ('39) and 8.7 mg. for eight children of Daniels et al. ('34), the mean value for these twenty-two children being 8.2 mg. per kilogram. On the basis of a somewhat different method for selection of the calcium-balance data reported in the literature, mean values of the same order of magnitude were obtained by Duckworth and Warnock ('42), namely, 138, 145, 159 and 129 mg. daily, for 2-, 3-, 4-, and 5-year-old children, respectively.

However the quantities of calcium retained by the subjects in this study are probably higher than can be accounted for

by growth needs alone. Mitchell, Hamilton, Steggerda and Bean ('45), basing their estimate on gains in total body weight and Shohl ('39) and Holmes ('45), in attempts to take into consideration the accelerated growth of the skeletal tissues during the first year of life, have concluded that the accretion of calcium during the preschool years is very small. The daily storages during the second, third, fourth, fifth and sixth years estimated by Mitchell et al. are 105, 70, 53, 50 and 60 mg., respectively, by Shohl 110, 90, 68, 105, and 138 mg., and by Holmes 117, 91, 88, 90 and 111 mg. Of the twelve children studied in this laboratory only two retained calcium in comparable quantities; i.e., the girl, M, whose daily retentions over a 6-week period averaged 106 mg. and the boy, G, whose retentions over a 7-week period averaged 84 mg. The considerably higher accretions found for the other subjects may be the result of a calcium impoverishment of the skeletal tissues present at the beginning of the metabolic studies and resulting from inadequate calcium feeding possibly instituted at birth (Stearns, '39). If this explanation of the discrepancy between the estimated and the observed values is valid, it is obvious that a period of liberal calcium feeding considerably longer than the 13-week period used in this study would have been necessary in order to secure calcium retentions relating solely to growth.

The range in values for the proposed daily requirement for dietary calcium of the seven boys is from 685 to 1165 mg. This range is much greater than that reported for five preschool girls (Outhouse et al., '39); for their total requirement, the extremes were 615 and 665 mg. or 32 and 41 mg. per kilogram. However, by applying to the data for the girls the method of assessment herein used, slightly different requirements than those published previously can be obtained. The new figures would be, for subjects B, M, J, P and S, respectively, 725, 855, 702, 535 and 679 mg. daily; they would average 699 mg. On the basis of such values, the per kilogram requirements would be 50.0, 60.6, 39.1, 30.2 and 35.5 mg. and would average 43.1 mg., whereas the per-centimeter requirements would be 7.8,

8.7, 7.1, 4.8 and 6.4, averaging 7.0 mg. Thus the requirements of the girls on a weight or height basis show a wider range than do those of the boys. The mean values for the two groups, however, are very similar; i.e., 45 and 43 mg. per kilogram and 7.8 and 7.0 mg. per centimeter for the boys and girls, respectively.

Since the non-milk foods available to the average American child would not, in the quantities usually eaten, provide as much calcium for the physiological calcification of skeletal tissues as children under experimental conditions have retained, an expression of the milk needs of the children of this study would seem pertinent. On the assumption that they could obtain daily, from the non-milk foods currently recommended for children of this age, approximately 300 mg. of calcium, the quantities of milk with which they would need to supplement such a dietary have been calculated and recorded in table 1. The resulting milk requirements range from 321 to 720 and average 478 gm. Six of the boys needed less than 1 pint daily; one who had a poor capacity to utilize dietary calcium could not have satisfied the calcium needs assigned to him by ingesting less than ¾ of a quart of milk.

SUMMARY

Seven preschool boys were subjected to a 40-week experiment during which calcium balances at different levels of calcium intake were determined for the purpose of assessing requirements for calcium. The values believed to be representative of the daily needs of the children were, in order of increasing age of the subjects, 150, 154, 137, 143, 148, 161 and 143 mg. On the basis of such retentions and at the rate at which each subject could utilize milk calcium, the daily requirements for dietary calcium have been computed, the resulting values being 843, 723, 685, 831, 1165, 826 and 803 mg. or 53, 44, 35, 50, 57, 37 and 40 mg. per kilogram, respectively. Assuming that children, under acceptable dietary conditions, could secure approximately 300 mg. of calcium daily from the non-milk foods which they eat and that such calcium is utilized

to the same extent as is that of milk, the requirements of six of the children would have been met by a milk supplement no greater than 2 cupfuls. One subject would have needed as much as ¾ of a quart.

ACKNOWLEDGMENT

The generous cooperation of Mrs. Charlotte Fitzgerald, superintendent, and members of the Board of Trustees of the Cunningham Children's Home, where this experiment was conducted, is herewith gratefully acknowledged as is that of Miss Nellie Ratcliffe, who prepared the food for the children, of Miss Charlotte Beard, who supervised their play and educational program and of Dr. J. B. Gillespie of the Carle Hospital Clinic, who examined them from time to time.

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